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MORPHOLOGICAL CHANGES IN REGENERATING CHELIPEDS OF THE BLUE CRAB *CALLINECTES SAPIDUS* RATHBUN AS INDICATORS OF THE PROGRESSION OF THE MOLT CYCLE.

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ABSTRACT A study of the morphological changes in the regenerating cheliped of the blue crab *Callinectes sapidus* Rathbun was conducted. Seven distinct morphological stages of limb bud development, designated G-stages, were categorized. These stages were correlated to Regeneration Index values (R-values), paddle molt stages, and the number of days to ecdysis. The relative utility of G-stages, R-values, and paddle molt stages in monitoring molt progression was evaluated. The G-stages and R-values can be used to monitor the relative progress of the molt cycle from intermolt to ecdysis. They are particularly useful in monitoring molt progress during intermolt, when no changes occur in the paddle, and in detecting the beginning of proecdysis when paddle staging is difficult. During late proecdysis, G-stages are reliable indicators of the time to ecdysis.

KEY WORDS Blue crab, *Callinectes sapidus*, molt cycle, chelipeds, regeneration.

INTRODUCTION

Brachyurans have the ability to automize limbs between the basi-ischium and coxa and to subsequently regenerate the lost appendages (Wood and Wood 1932). This response is associated with physical damage to the limb and is generally thought to be an adaptive advantage to the crab. The regenerating limbs grow in a folded position within a cuticular sac (Adiyodi 1972). They unfold and greatly expand during ecdysis. Regeneration and the molt cycle are interrelated because limb regeneration is complete at ecdysis. Skinner and Graham (1972) demonstrated the existence of a critical period (stage D_1) in the molting cycle of the land crab, *Gecarcinus lateralis* H. Milne Edwards, before which limbs must be removed if they are to be successfully regenerated. Thus, growth and regeneration are coordinated with the molting process. The effects of limb regeneration on the molt cycle have been studied in a number of crustaceans (Bliss 1956; Hodge 1956; Durand 1960; Rao 1966, 1978; Fingerman and Fingerman 1974; Holland and Skinner 1976; Weis 1976; Hopkins 1982). Skinner and Graham (1972) found that the loss of six to eight limbs including chelipeds triggered precocious molts in the blue crab, the green crab *Carcinus maenas* (Linnaeus) and the fiddler crabs *Uca pugnax* (S.I. Smith) and *U. pugilator* (Bosc). Regeneration, therefore, is not only dependent upon molting, but can also affect the length of time from ecdysis to ecdysis.

Bliss (1956) divided the regeneration process into the following descriptive stages. After an initial lag period following autotomy during intermolt, basal growth occurs that produces a small limb bud. A basal growth plateau, a period of no growth, may follow the emergence of the limb bud. During proecdysis, however, the regenerating limb begins to grow rapidly, followed by another period of slowed growth just prior to ecdysis, i.e., the terminal growth plateau. Similar patterns of limb regeneration have been observed in a variety of other crustaceans (Durand 1960; Skinner 1962; Passano and Jyssum 1963; Kurup 1964; Stevenson and Henry 1971).

This study of cheliped regeneration in the blue crab was conducted to describe the morphological changes of chelipeds during cheliped regeneration and to relate the morphological features to the relative growth rate of the chelipeds and to the progression of the molt cycle. Other workers (Adiyodi 1972; Hopkins 1982) have used the postautotomy development of walking legs to monitor crab molt cycles. We studied cheliped development as part of an ongoing study of the effects of autotomy of both chelipeds on molting in the blue crab. Cheliped autotomy may be a means of inducing, synchronizing, and monitoring molting in the mass culture of hard, intermolt crabs for the production of soft-shell crabs. This investigation resulted in (1) a general description of the pattern of cheliped regeneration in juvenile blue crabs, (2) the categorization of morphological changes in the regenerating cheliped and the definition of seven recognizable stages in limb development, and (3) a description of the relationship between the progression of cheliped regeneration and the molt cycle.

MATERIALS AND METHODS

Crabs were collected from the Rigolets, a brackish-water pass between lakes Borne and Pontchartrain, east of New Orleans, Louisiana. Crabs were captured using baited nets, baited lines, and dip nets. Only apparently healthy crabs with all appendages intact were used in this study.

Crabs were placed in closed, recirculating seawater systems in the laboratory. Functional components of the systems used in this study were described by Perry et al. (1979). Our systems consisted of holding tanks, filters, and pumps. The holding tanks were circular, plastic, children's wading pools, 120 cm in

diameter and 20 cm in depth. A hose placed in the sides of the pools permitted drainage into a filter and maintainence of the water depths between 6.0 and 7.0 cm.

The filter system functioned as a composite of biological and mechanical processes. A 75-L (20-gal) plastic garbage can, 57 cm in height and 46 cm in diameter, was filled to a height of 33-43 cm with medium-grade crushed oyster shell (Pilot Brand, Special Pullet). The filtered water was collected in CPVC pipe which ran through the shell, and was drawn from the filter by a 0.025-hp, horsepower pump (Little Giant, Model 2E-38N). Water was returned to the holding tanks at a rate of approximately 70 $ml \cdot sec^{-1}$. The total volume of water in the system was approximately 350 *l*. Salinity was maintained at 15 to 20 $^{0}/_{00}$ using commercial sea salts (Rila Marine Mix). Water in the pool was aerated vigorously, the temperature was maintained between 21° and 24°C, and crabs experienced a 12:12-h, light:dark photoperiod, with the light period beginning at 6 a.m. The light sources were cool white fluorescent blubs (General Electric Corp.). Nitrite levels never exceeded 0.1 $mg \cdot L^{-1}$, which was below the level found by Manthe et al. (1984) to be toxic to molting blue crabs held in closed, recirculating systems. Ammonia levels were maintained below 1.0 $mg \cdot L^{-1}$ total ammonia, the level below that which Lakshmi et al. (1984) recommended for shedding blue crabs.

Five holding tanks were used in this study. A total of 65 experimental crabs were held in these tanks. Populations in each tank were matched as well as possible according to size, sex, and number.

The carapace width was determined as the distance between the tips of the ninth lateral spines. The carapace width of crabs used in this study from 40 to 132 mm. The length of the regenerating cheliped was measured under a dissecting microscope with ocular micrometer or with a metric ruler. A Regeneration Index (Bliss 1956) was calculated to compare the regenerating chelipeds of crabs of different carapace widths. The equation used for the calculation was:

$$\text{Regeneration Index (R-value)} = \frac{\text{Bud length X 100}}{\text{Carapace width}}$$

Cheliped autotomy was induced by crushing the merus with pliers. The limb autotomizes at the basi-ischium and coxa articulation (Wood and Wood 1932). In all experimental crabs, both chelipeds were autotomized and no other limbs were affected.

Molt staging was performed by the examination of the last two segments of the fifth pereiopod, or paddle leg, according to the common method of peeler classification described by Perry et al. (1979), Johnson (1980), Otwell (1980), and Oesterling (1984). A comparison of peeler classification with Drach's (1939) substages of proecdysis is presented in the discussion section of this paper. Four recognizable substages of proecdysis were observed in this study. The first indication of premolt is the appearance of an amber-colored zone forming along the outer edge of the paddle tip, similar to that observed in the uropods of the porcelain crab *Petrolisthes* by Kurup (1964) and in the pleopods of the lobster *Homarus* by Aiken (1973). There is also a localized chromatophore displacement with the formation of this amber-colored zone (Aiken 1973). The second stage is marked by noticeable apolysis. A gap forms between the new and old exoskeleton due to epidermal retraction. A double border with a clear to grayish band along the new epidermal edge is observed. This stage is commonly referred to as the *white-line stage*. The third stage, the *pink-line* stage, shows a pink color in the gap between the retracting epidermis and the old exoskeleton. With the secretion of the new exocuticle, a distinct red line runs between the new epidermis and the old exoskeleton. This last stage is known as the *red-line stage*, and marks the last discernable change in the paddle leg prior to ecdysis.

Limb bud growth was monitored every 2 to 3 days from the time of cheliped removal until ecdysis. Measurements of the regenerating cheliped were recorded at each observation. The R-values were then calculated for each cheliped of each crab. Thirty-nine of the 65 crabs were staged for molt by examination of the paddle leg. The morphological characteristics of the limb bud were also recorded.

RESULTS

Seven distinct morphological stages of the regenerating cheliped were categorized (Figure 1). These stages have been designated as *G-stages*. The first of these stages, G_1, is marked by a bulge forming from the plane of autotomy. The second stage, G_2, is noted by the appearance of a papilla forming from the bulge. As the papilla grows larger, it bends backward at an angle of approximately 45°. This stage is defined as the G_3 stage. In the G_4 stage, the segments of the regenerating bud become more defined. Segments of the cheliped are noticeable, and the limb bud becomes gray. The G_5 stage is characterized by a change in color from gray to a solid white on the tips of the developing dactylus and propodus. White streaks appear on the remaining gray portions of the propodus. In the next stage, G_6, the dorsal surface of the limb becomes brown, and teeth appear on the propodus and dactylus. The last stage, G_7, is categorized by a general darkening of the bud. The bud appears reddish-brown, except for the dactylus which is dark red.

For each G-stage, the mean R-value and the mean number of days to ecdysis were determined (Table 1). Each G-stage had a fairly discrete range of R-values associated with it, until the limb bud reached the G_6 and G_7 stages when overlapping of the R-values occurred. The number of days until ecdysis for each G-stage varied. This variation was highest for G_1 and G_2, less for G_3, G_4, G_5, and G_6, and least at G_7. The degree of overlap in number of days until ecdysis was high between G_1 and G_2, and G_3 and G_4. Less overlap occurred between G_2 and G_3, G_4 and G_5, G_5 and G_6, and G_6 and G_7. When a crab limb reached the G_7 stage, ecdysis occurred within 4 days.

G-stages and R-values associated with each paddle molt-stage from intermolt to ecdysis are presented in Table 2. Although the numbers assigned to G-stage are not algebraically additive,

treating these numerical designations as actual numbers provided a convenient means of expressing the mean morphological condition of the regenerating cheliped. The mean G-stage and R-value, and the mean number of days to ecdysis for each of the paddle molt stages were determined, except for intermolt, where the maximum G-stage obtained, the maximum R-value obtained, and the minimum number of days to ecdysis were determined. Mean values for G-stages increased as the crabs progressed through the paddle molt-stages. There was variation and some overlap in the range of G-stages that were associated with each paddle stage. The least overlap occurred between maximum values that were associated with intermolt and values associated with the amber-zone stage which indicated the beginning of pro-ecdysis. Although G-stages can be used to monitor progression in the molt cycle, these data which included carapace sizes that ranged from 40 to 132 mm did not show a clear correlation of a particular G-stage with a particular paddle stage. The R-values also showed considerable variation with paddle stages. The least amount of variation was between the maximum values that were associated with intermolt and mean values associated with the amber-zone stage. The mean number of days until ecdysis for each paddle molt-stage also varied, until the red-line stage. At this point, the red-line stage is a reliable indicator of imminent molt.

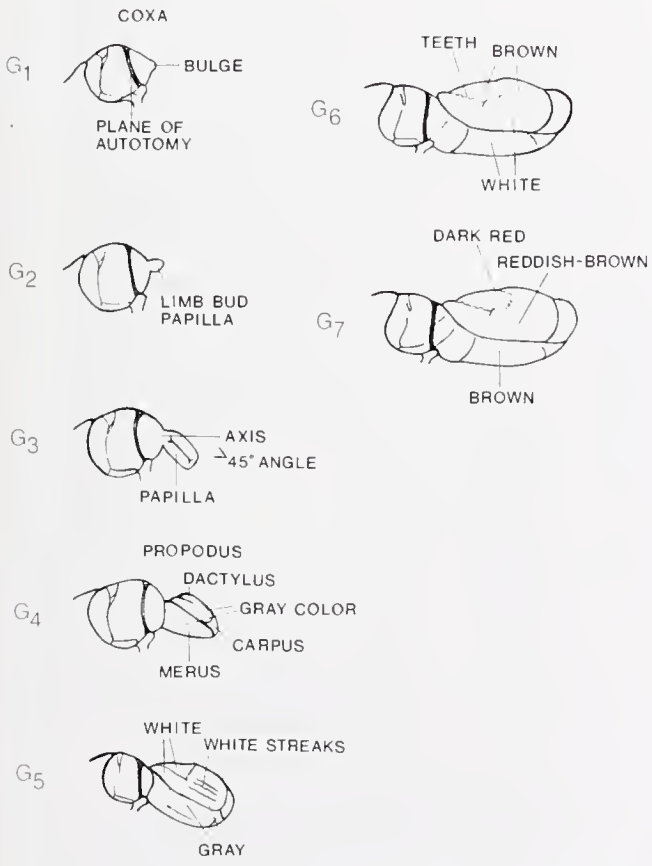

Figure 1. Diagram of the seven morphological stages (G-stages) of the regenerating cheliped, as development proceeds from autotomy to ecdysis.

TABLE 1.

A comparison of the mean R-value and the mean number of days from autotomy to ecdysis determined for each G-stage observed.

G-STAGES	MEAN R-VALUE*	MEAN NUMBER* OF DAYS TO ECDYSIS
G_1 (n=6)	2.9±0.7	21.8±6.7
G_2 (n=27)	4.2±0.8	19.5±7.0
G_3 (n=29)	6.2±1.0	11.9±3.9
G_4 (n=22)	8.7±1.6	11.2±4.2
G_5 (n=34)	12.8±2.7	7.4±3.6
G_6 (n=39)	16.2±2.7	5.4±3.1
G_7 (n=34)	17.5±2.2	2.7±1.8

*(Value = mean ± 1 standard deviation)

The relationship between the carapace width and the number of days from autotomy to ecdysis is shown in Figure 2. These data show that the length of regeneration period and the variation in the length of that period increase with crab size. Crabs with a carapace width of 40 to 65 mm had the least variation, crabs from 66 to 100 mm had more variation, and those above 100 mm had the greatest variation.

To compensate for the varying lengths of time required for cheliped regeneration (Figure 2), the regeneration time was standardized by converting days from autotomy to a percentage of the regeneration period (Hopkins 1982). The percent of the regeneration period is the time of observation after autotomy divided by the total regeneration period (days from autotomy

TABLE 2.

A comparison of the mean G-stage, the mean R-value, and the mean number of days from autotomy to ecdysis determined for each paddle stage observed.

PADDLE MOLT STAGES	MEAN G-STAGE*	MEAN R-VALUE*	MEAN NUMBER* OF DAYS TO ECDYSIS
INTERMOLT** (n=32)	3.1±0.7 (maximum)	6.6±1.8 (maximum)	11.3±2.1 (minimum)
AMBER-ZONE (n=39)	4.7±0.9	11.0±2.9	9.1±2.8
WHITE-LINE (n=36)	5.4±0.8	14.4±2.7	5.5±1.4
PINK-LINE (n=37)	6.3±0.6	16.7±2.5	3.9±1.6
RED-LINE (n=29)	6.8±0.4	17.5±2.5	1.8±1.0

*(Value = mean ± 1 standard deviation)

**(For intermolt the maximum G-stage and R-value, and the minimum number of days to ecdysis was determined.)

to ecdysis). The regeneration period was partitioned into ten-percentage-point intervals and the R-values that were associated with each interval were plotted. These results are presented in Figures 3. The R-values showed considerable variation within each interval of the regeneration period. The rate of R-value increase may be partitioned into two slopes. Following an initial lag period, there was an increase in the rate of growth between the 10-19% and 20-29% intervals. Between the 50-59% and 60-69% intervals an additional increase in growth rate occurred. This second change in slope occurred between mean R-values of approximately 7 and 10 and may be correlated with the passage of the animals from intermolt into proecdysis (see DISCUSSION section). During the final ten-percentage-point interval of the regeneration period, there was little linear growth as the animals prepared for ecdysis. A wide range of R-values (12.3 to 22.7) was still present for mature limb buds. This result indicated that much of the variation in R-values may be caused by individual variation in cheliped size.

Figure 4 presents the patterns of cheliped regeneration, as expressed by R-values, in seven representative crabs of different sizes and of both sexes. Data from four juvenile crabs, selected from a larger sample that was used to construct Figure 2, illustrate the individual differences in the rates of regeneration. For juvenile crabs, there was usually a lag period followed by a relatively linear rate of increase in R-values until a few days before ecdysis. In the cases where R-values were examined in the last three days before ecdysis, there was little or no increase in R-values during that period. Long growth plateaus during the regeneration period were not observed for crabs that successfully molted and regenerated chelipeds. The patterns shown for juvenile crabs in Figure 4 are typical examples of the patterns shown by juvenile crabs in the larger sample (Figure 2). Generally the rate of cheliped growth (as measured by R-values) decreased with increasing crab size. Included in Figure 4 are data for adult females. These females would not be expected to molt after cheliped autotomy because adult females very rarely molt after sexual maturity (Abbe 1974). Adult females reached a maximum R-value of ~4 and developed to the G_3 stage. Large males (> 140 mm) reached a maximum R-value of ~7 and developed to the G_4 stage. Large male and mature female crabs usually died after 100 days without showing any paddle molt-signs of proecdysis.

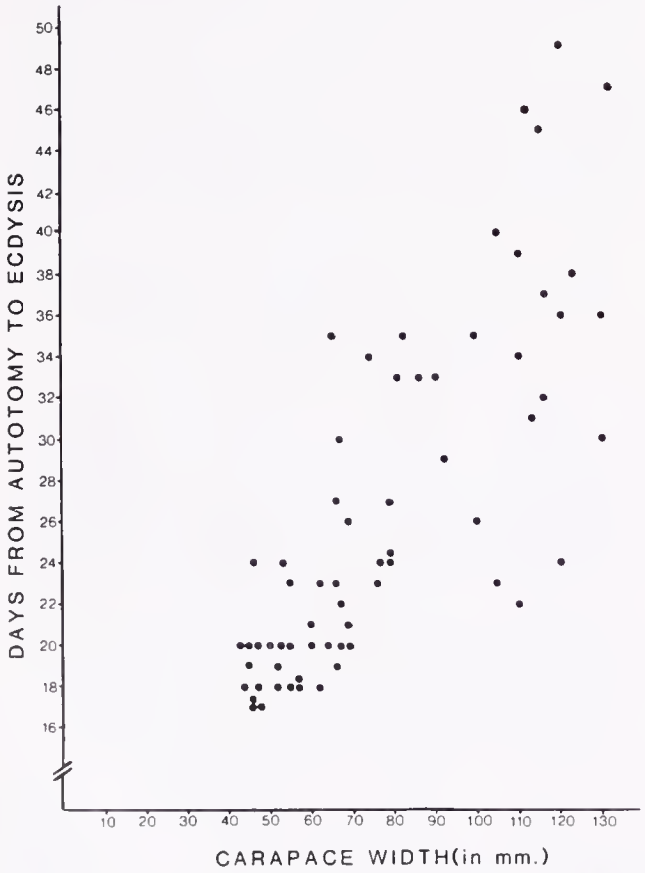

Figure 2. The relationship between the carapace width of 65 crabs and the number of days from autotomy to ecdysis (r = 0.79).

DISCUSSION

These studies have demonstrated that morphological changes in regenerating chelipeds termed *G-stages* (Figure 1 and Table 1) and R-values (Figure 3) can be used to monitor the progression of the molt cycle in the blue crab. The relationships between paddle molt-stages, G-stages, R-values, and days to ecdysis (Table 2) show the relative utility of G-stages and R-values as indicators of events in the molt cycle.

Although G-stages require subjective interpretation, they can be assessed without the measurements required of R-values, and are easier to determine than paddle stages. Limb-bud development begins while crabs are in intermolt and continues through premolt; therefore, G-stages could be used to monitor progress to ecdysis during intermolt and the transition between intermolt and the early stages of proecdysis (Table 2). Based on paddle staging, the mean maximum G-stage for intermolt in juvenile crabs was 3.1±0.7 (Table 2). This maximum value for intermolt is consistent with the limb growth response demonstrated by adult females (Figure 4). In adult females, which would not be expected to enter proecdysis (Abbe 1974), regenerating limbs never developed past the G_3 stage. The G_7 stage in juvenile crabs can be used to predict ecdysis. This method, however, does not appear to be more accurate than the paddle red-line stage.

Each G-stage had a distinct range of R-values, except for stages G_6 and G_7 when R-values changed very little (Table 1). During G_6 and G_7 chelipeds continued to change in morphology late in the molt cycle when there was little linear growth of the limb bud. The G-stages, therefore, are better than R-values in predicting ecdysis late in the molt cycle.

The R-values, which are less subjective than G-stages or paddle stages, can also be used to monitor the transition from intermolt to proecdysis. Data in Table 2 indicate mean maximum R-values of 6.6 associated with intermolt and a mean value of 11.0 with the beginning of proecdysis; however, regenerating

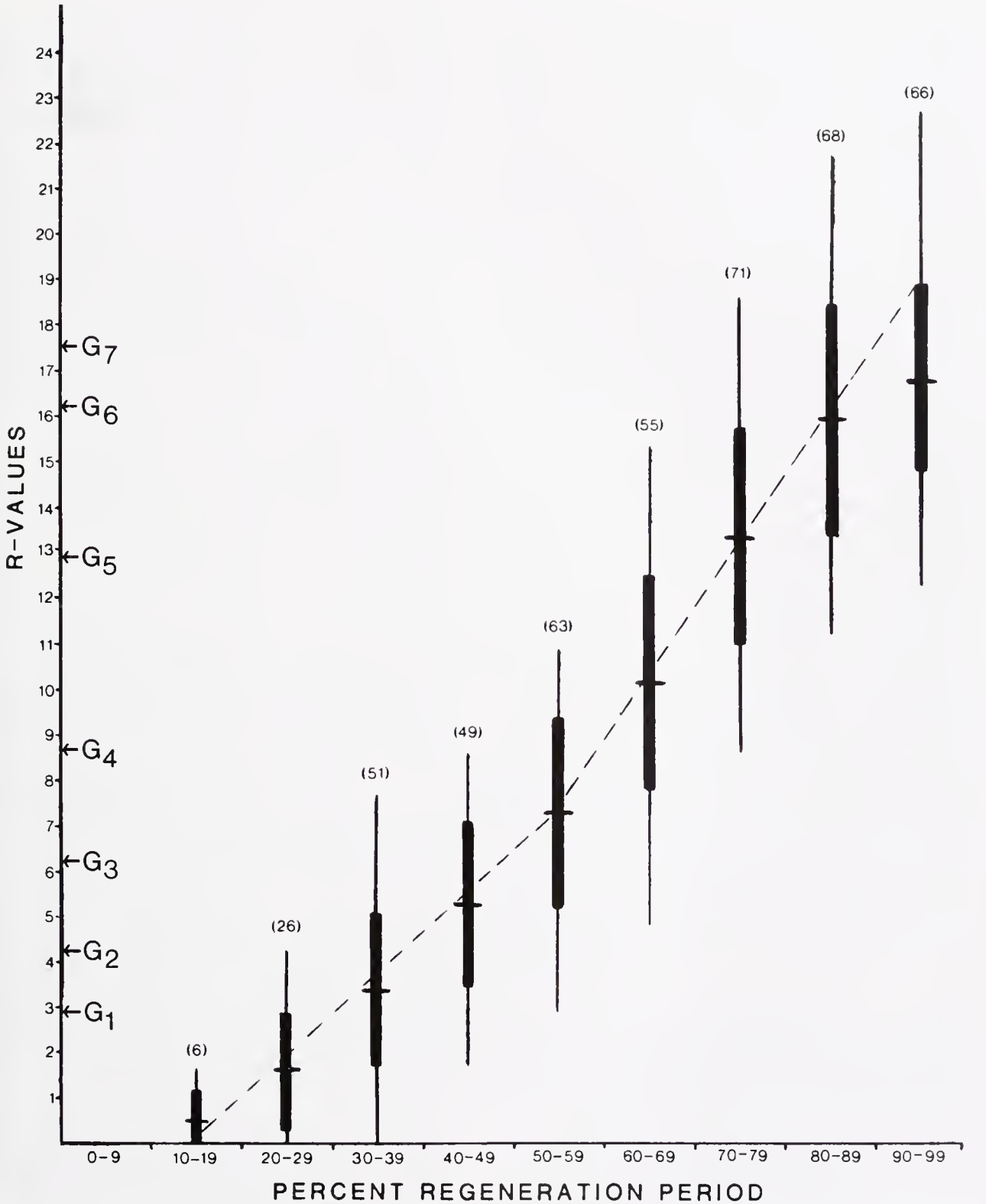

Figure 3. The relationship between percent of regeneration period and R-values. The percent of the regeneration period is the time of observation after autotomy divided by the total regeneration period (days from autotomy to ecdysis). The horizontal lines are the mean R-values, the vertical lines are the ranges, and the rectangles are plus and minus 1 standard deviation. Each datum point represents the mean of the R-values for the designated 10% range of the regeneration period. The broken lines indicate a visual approximation of two possible slopes, based upon the mean R-values. The G-stages are indicated on the vertical axis corresponding to the mean R-value that were determined for each from Table 1. The number in parentheses indicate the number of observations.

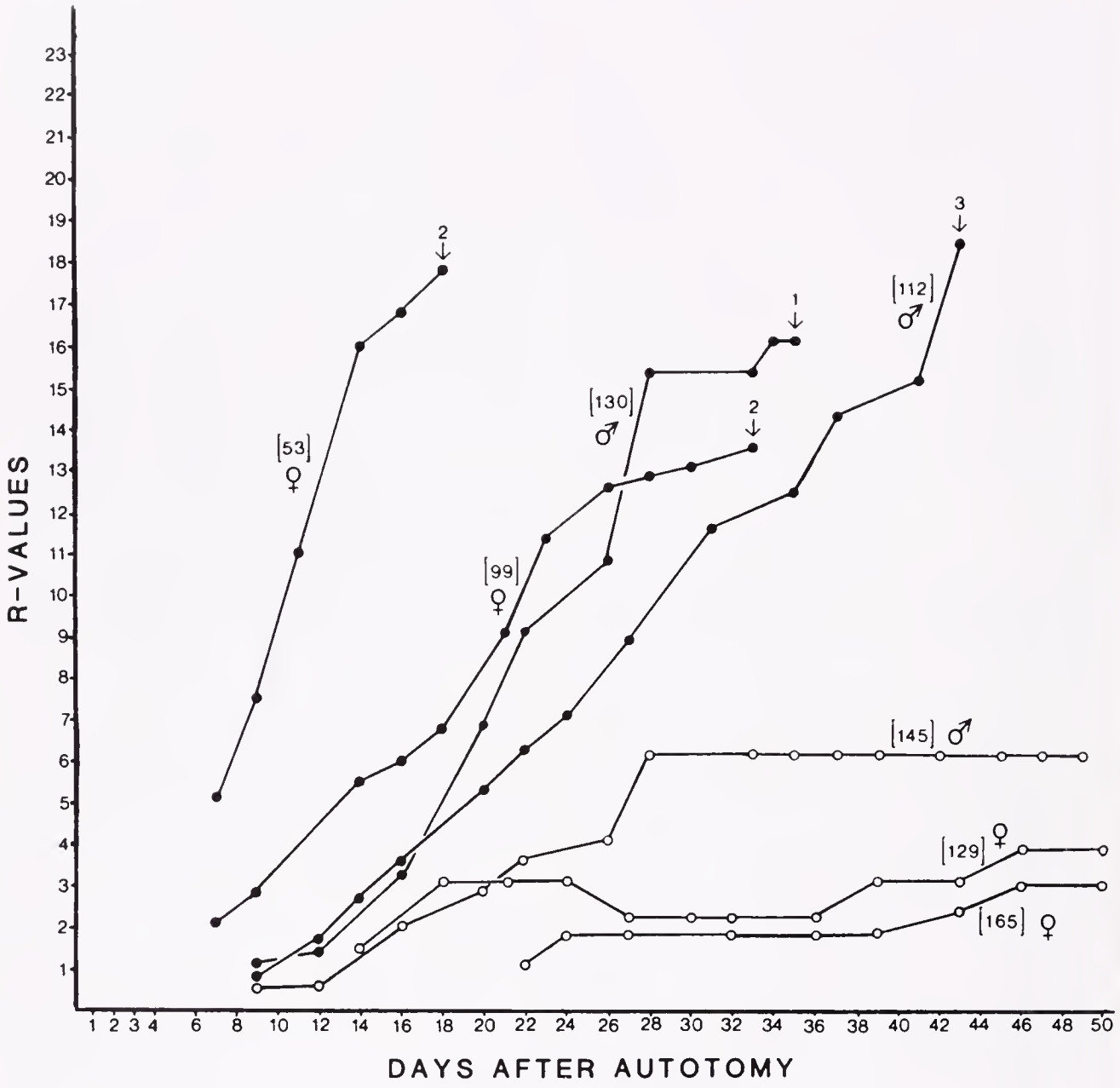

Figure 4. The relationship between the number of days after autotomy and the R-values determined for seven representative crabs of different carapace widths. Dark circles indicate observations on juvenile crabs that successfully regenerated the chelipeds and molted. The open circles are observations on a large male and two adult females that did not molt within the period of observation (100 days). The arrows designate the last R-value determination before ecdysis for each crab. The numbers above the arrows indicate the number of days until ecdysis for the individual crab. The carapace width (in mm) of each crab is indicated in brackets. The sex of each crab is also indicated.

limbs of adult females reached maximum R-values of only 3.9 (Figure 4). The reason for this difference in maximum R-values that we obtained is unknown. Regenerative growth is probably arrested earlier in animals which will not enter proecdysis.

Cheliped limb buds of large males (> 140 mm) reached R-values that ranged from 6.1 to 7.2 and averaged 6.6, and all developed to the G-4 stage without further changes until death. These R-values and G-stages were in the general range of R-values (6.6 ± 1.8) and G-stage (3.1 ± 0.7) that were associated with the transition between intermolt and proecdysis in juvenile crabs (Table 2). They did not develop paddle molt-stages that were characteristic of proecdysis, but did develop maximum R-values and G-stages that were higher than maximum values for adult females. These large males may have reached a basal growth plateau that was associated with long intermolt periods. We assume that under better culture conditions, or in nature, cheliped regeneration would have continued, culminating in ecydsis. A basal growth plateau has been reported in other anec-

dysic crustaceans (Bliss 1956; Durand 1960; Passano and Jyssum 1963; Stevenson and Henry 1971).

The time from autotomy to ecdysis increased with size of the crab (Figure 2). Churchill (1921) and Tagatz (1968) found that the time between molts became progressively longer as size increased. The relationship between time from autotomy to ecdysis and size may be due to the increasing length of the intermolt period with increasing animal size. The larger variation in regeneration period that was associated with size (Figure 2) may result from variation among larger crabs in the time from their previous molt to the time of autotomy. Larger crabs which had molted shortly before autotomy may have taken longer to regenerate chelipeds than crabs which were near proecdysis when the chelipods were autotomized. Variation may also have been influenced by stress of the culture system. Large crabs that were held for more than 100 days in the system usually died of undetermined causes.

The growth of cheliped limb buds was continuous in juvenile crabs (Figures 3 and 4) and did not show the basal growth plateau. Although large juvenile crabs (carapace width 100-132 mm) showed considerable variation in time from autotomy to ecdysis (Figure 2), growth was continuous but occurred at different individual rates (Figure 4). The crabs which were closer to proecdysis when autotomy occurred may have had a faster rate of regeneration than those which had recently entered intermolt.

A broad range of R-values was found throughout the regeneration period (Figure 3). This range was influenced by individual variation in limb-bud size, as evidenced by the large range in R-values present before ecdysis (Figure 3), and to individual variation in the rate of limb-bud development (Figure 4). A decrease in the rate of growth, as measured by R-values, occurred at the end of the regeneration period (Figures 3 and 4). The G-stages, however, which are based on other morphological characters, continued to change during this period (Table 1). A slight increase in the rate of linear growth occurred between 50-59% and 60-69% of the regeneration period (Figure 3). This increase occurred as mean R-values increased from 7.3 to 10.2. These values are close to the mean R-values of 6.6 associated with intermolt and 11.0 associated with the beginning of proecdysis (Table 2). This apparent rate increase may be related, therefore, to this transition between intermolt and proecdysis. Bliss (1956), Adiyodi (1972), and Hopkins (1982) also reported an increase in the rate of limb regeneration upon entering proecdysis.

The relationship between the paddle molt-stages and the substages of proecdysis, as described by Drach (1939), has not been clearly established for the blue crab. Although Johnson (1980) did not note the amber-colored zone in the blue crab, Aiken (1973) observed it in lobster *Homarus*, and Kurup (1964) observed it in the procelain crab *Petrolisthes*. Aiken (1973) equated the amber zone to the D_0 stage of Drach (1939). Johnson stated that the epidermis of the crab still remains firmly attached to the cuticle during stage D_0. The beginning of apolysis was viewed by Skinner (1962) and Johnson (1980) as occurring during stage D_1. Aiken (1973), however, considered apolysis to be in stage D_0 and regarded this stage as a transition phase between obvious intermolt and obivous premolt. It is during stage D_0 in lobsters that the period of anecdysis, or pause between molts, occurs (Aiken, 1973). The irreversible transition from intermolt to proecdysis in *Homarus* takes place between D_0 and D_1. This might explain the higher R-values and G-stages found in large male crabs compared to mature females. Those males may have actually entered stage D_0 and into a period of anecdysis. We assume that the white-line stage is the product of the pronounced separation of the epidermis from the old cuticle, and occurs at stage D_1. Johnson (1980) stated that the appearance of the red-line stage, indicating the secretion of the exocuticle, occurs during the later part of stage D_2. Other researchers (Skinner 1962; Stevenson et al 1968; and Aiken 1973) have also categorized the secretion of the exocuticle as being in stage D_2. The pink-line stage has not been related to a particular Drach stage, presumably because it may only be a transition in the secretion of the exocuticle. Johnson (1980) stated that indications of stage D_3 are lacking in the epidermis of the blue crab.

The present study demonstrates that cheliped limb-bud regeneration can be used to monitor the molt cycle in the blue crab from periods of intermolt to ecdysis. Although G-stages and R-values show considerable variation, they can be used to determine the relative progress of blue crabs toward ecdysis. They are particularly useful in monitoring progress during intermolt when there is no change in the paddle margin, and early proecdysis when the use of paddle staging is difficult. During the late stages of proecdysis, G-stages are reliable indicators of the time to ecdysis. These results could be employed in future studies of the molt cycle of the blue crab and in the short-term aquaculture of hard, intermolt crabs to soft crabs for commercial production.

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RESEARCH NOTE

RELATIVE BLUE CRAB ABUNDANCE IN TEXAS COASTAL WATERS

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ABSTRACTS Populations of the blue crab *Callinectes sapidus* Rathbun were monitored from December 1977 to November 1981 by the Texas Parks and Wildlife Department (TPWD) using 18.3-m bag seines in fishery-independent sampling in Galveston, Matagorda, San Antonio, Aransas, and Corpus Christi bays and the upper and lower Laguna Madre. Catch rates were analyzed for differences using a three-way analysis of variance examining years, seasons, and bay systems. Mean annual coastwide rates were found to vary significantly ($P \leq 0.05$) among years. Catch rates increased from 1978 to 1979 and stabilized through 1981. Greatest relative abundance of blue crabs occurred during spring and summer. Significant variations in annual coastwide catch rates were detected. These variations indicate that stratifying data into one high-catch season (spring-summer) and one low-catch season (fall-winter) would provide more precise estimates of relative abundance than yearly analyses thereby aiding in future management decisions and the analysis of the impact of such decisions.

KEY WORDS: Blue crab, abundance, Texas, *Callinectes sapidus*

INTRODUCTION

The blue crab *Callinectes sapidus* Rathbun is found along the entire Texas coast and supports the third largest commercial fishery in the state, following shrimp and oysters. Annual landings of hardshell blue crabs from 1977-1981, averaged 3.6 x 10^6 kg and were valued at $2.0 million dockside (Hamilton 1983).

Stock assessment of blue crabs through fishery-independent sampling is necessary for effective evaluation and implementation of management decisions (Hegen et al 1983; McEachron and Green [1984]). During 1977, Texas Parks and Wildlife Department (TPWD) initiated a routine bag-seine sampling program that was designed to obtain statistically reliable catch rate data for juvenile finfishes and shellfishes including blue crabs (Hammerschmidt 1982). The objective of this study was to determine if there were significant differences in mean bag-seine catch rates of blue crabs among years, seasons, and geographical location between December 1977 and November 1981.

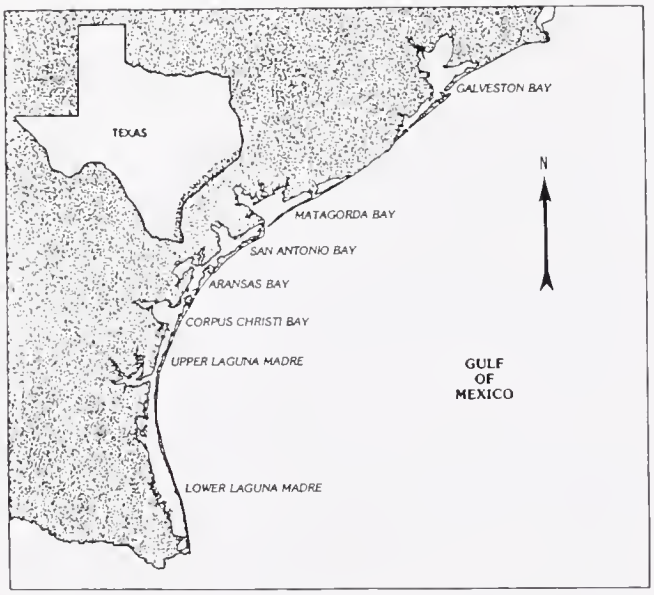

Figure 1. Texas bays sampled in this study.

MATERIALS AND METHODS

Samples were collected between December 1977 and November 1981 with bag seines in Galveston, Matagorda, San Antonio, Aransas and Corpus Christi bays and the upper and lower Laguna Madre (Figure 1). Bag seines were 18.3 m long and 1.8 m deep with 19-mm stretched nylon multifilament mesh in the lateral wings and 13-mm stretched multifilament mesh in the central bag. Six different shoreline stations were sampled each month in each bay except during June 1978 when no samples were collected and beginning in November 1981 when ten samples were collected in each of the above bays. Stations were randomly selected from a list of $\leq$ 100 sample stations compiled for each bay (Hegen 1982). One-half of the stations were sampled during the first two weeks and one-half during the last two weeks of each month. Each sampling week extended from sunset Sunday to sunset the following Sunday. Stations were sampled during daylight hours.

A bag-seine sample was collected by pulling an extended seine parallel or perpendicular to shore for a distance of not

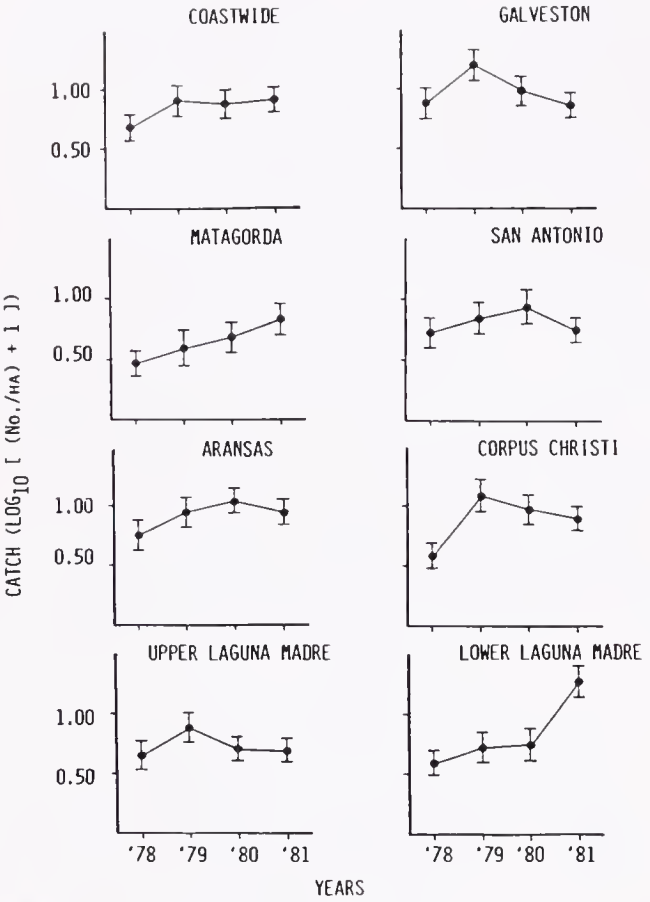

Figure 2. Mean annual bag-seine catch rates ($\log_{10}$[(No./ha) + 1] ± 1 SE) of blue crabs in individual Texas bays and coastwide (n = 18).

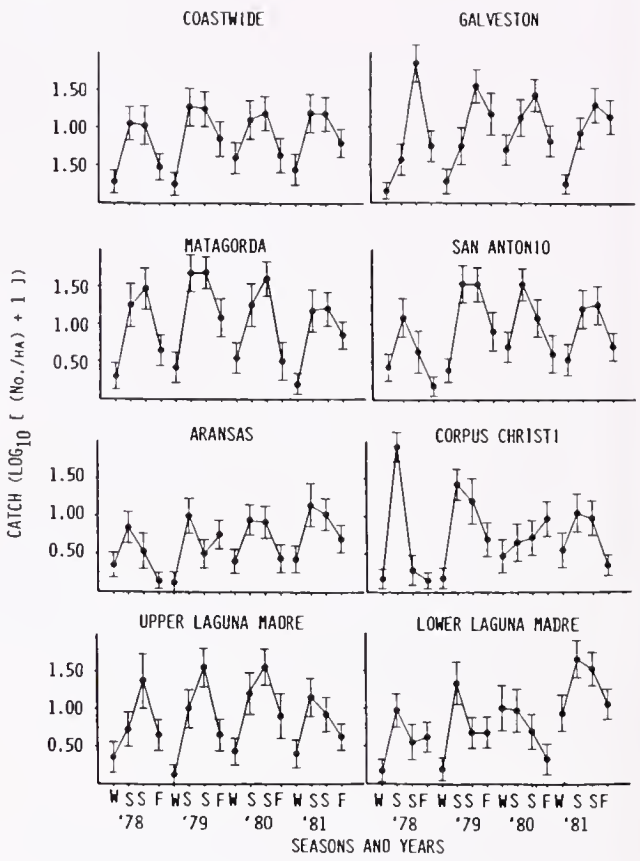

Figure 3. Mean seasonal bag-seine catch rates ($\log_{10}$[(No./ha) + 1] ± 1 SE) of blue crabs in individual Texas bays and coastwide by year (n = 18).

less than 15.2 m and not more than 30.5 m. The rectangular surface area sampled was estimated using the distance pulled and the length of extension of the bag seine.

Catch rates were calculated by dividing the total number of juvenile and adult blue crabs caught by the total area (in hectares) sampled at each station. Coastwide catch-rate estimates were calculated by weighting individual bay values by the total amount of shoreline present in the respective bay (Matlock and Ferguson 1982). The term coastside in this study represents combined data from the seven bays listed above. Values were reported to the nearest 0.01/ha. Monthly data were combined into four seasons, each containing three months; winter (December-February), spring (March-May), summer (June-August) and fall (September-November). This arrangement was chosen on the basis of current knowledge of the life history of the blue crab in Texas as provided by Daugherty (1952) and More (1969).

Bag-seine catch rates were examined for significant differences ($P \leq 0.05$) among years using a three-way analysis of variance (Sokal and Rohlf 1969). The three factors analyzed were years, seasons, and bays. Catch rates were transformed to reduce the level of unequal variances using a common logarithmic transformation:

$$t_i = \log_{10}[(x_i/ha) + 1]$$

Where t_i = value of ith catch rate after transformation,
x_i = catch of ith sample, and
ha = total hectares sampled.

Sums of squares for summer 1978 and fall 1981 were estimated using n = 18 to equalize sample sizes for all seasons and years. Since sums of squares were estimated, confidence limits are not exact; however, significant differences were inferred whenever any two of these confidence limits failed to overlap.

RESULTS

The relative, coastwide abundance of blue crabs in Texas bays generally increased from 1978 to 1979 and then remained constant through 1981. Annual mean coastwide, bag-seine catches were significantly different among years ($F = 5.998$; d.f. = 3, 1918; $P \leq 0.05$) with catches in 1978 significantly lower than the other years (Figure 2). This pattern was, however, not consistent among bays (Figure 2) as reflected by a significant two-way interaction term for years and bays ($F = 2.176$; d.f. = 18, 1918; $P \leq 0.05$). Constant catch rates from 1979 through 1981 were not evident for the lower Laguna

Madre where an increase occurred during 1981, nor for Galveston and San Antonio bays and the upper Laguna Madre where 1981 catches were similar to those of 1978 (Figure 2).

Coastwide blue crab abundance was generally greater during spring and summer than during fall and winter (Figure 3). A significant difference in mean bag-seine catches was found among seasons ($F = 40.463$; d.f. = 3, 9; $P \leq 0.05$). This pattern was, however, not consistent among years or bays as reflected by significant two-way interaction terms for years and seasons ($F = 1.944$; d.f. = 9, 1918; $P \leq 0.05$) and for seasons and bays ($F = 2.768$; d.f. = 18, 54; $P \leq 0.05$). Coastwide 1979 fall catch rates were different from 1979 winter catch rates, whereas during all other years, fall catches were similar to winter catches (Figure 3). Fall catches were highly variable within bays among years as reflected by fall 1978 data from San Antonio, Aransas, and Corpus Christi bays as well as fall 1980 from the lower Laguna Madre (Figure 3).

DISCUSSION

The ability to detect differences in blue crab abundance is essential for understanding reasons for fluctuations and for predicting future year-class strengths (Gulland 1969). Significant trends in coastwide annual and seasonal blue crab abundance were detected through the use of fishery-independent sampling with bag seines; however, to obtain the most accurate prediction, it is necessary to estimate abundance with maximum precision. Changes in seasonal abundance of blue crabs found during this study were consistent with those reported by Daugherty (1952), Hammerschmidt (1982, 1983), King (1971), and Perry et al (1984). Estimating abundance during a high-catch season (spring-summer) and a low-catch season (fall-winter) separately would provide a more precise estimate of relative coastwide abundance than pooling data for the entire year. This has been demonstrated for other estuarine species by Hegen et al. (1983) and McEachron and Green (1984).

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I should like to thank each of the shellfish and finfish teams that helped collect the samples during this study. Special thanks go to Gary C. Matlock, C.E. Bryan, and Albert W. Green whose patience and understanding guided me through the completion of this report. Thanks also go to Roy Johnson, Ed Hegen, and Tom Heffernan for reviewing the manuscript. This study was carried out with partial funding from Federal Aid P.L. 88-309, Project (2-385-R).

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IMPORTANT VARIABLES IN THE DEFINITION OF EFFECTIVE FISHING EFFORT IN THE TRAP FISHERY FOR THE BRITISH COLUMBIA PRAWN *PANDALUS PLATYCEROS* BRANDT

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ABSTRACT In 1983 and 1984, three experiments were carried out to identify variables which should be accounted for in the definition of effective fishing effort in the trap fishery for the British Columbia prawn *Pandalus platyceros* Brandt. Soak time, depth, trap design, and entrance size were factors which significantly affected catch rates. Through a process of evaluating these and other variables it will be possible to develop a standarization algorithm which will permit accurate interpretation of catch-and-effort information for stock assessment.

KEY WORDS *Pandalus platyceros*, trap, effort standardization

INTRODUCTION

Measurement of effective fishing effort is essential when developing a fisheries catch-and-effort data base for stock assessment. Effective fishing effort is defined by Ricker (1975) to be the adjusted measurement of effort such that each increase in the adjusted unit causes a proportional increase in the instantaneous rate of fishing mortality. The measurement of effective fishing effort in crustacean trap fisheries is a complex task which Morgan (1979) pointed out is influenced by: (1) the fishing power of the gear, (2) the time during which the gear is actively fishing, (3) the distribution of animals and fishing effort, and (4) the vulnerability of the animal to the type of gear.

The trap fishery for the British Columbia prawn *Pandalus platyceros* started prior to 1914 (Butler 1980) with incidential catches reported from as early as 1887 (Mowat 1888). Since 1979 the fishery has undergone a rapid increase in effort from ~50 vessels to over 300 vessels in 1984. The vessels range in size from 5 to 35 m and work between 40 and 1500 traps. The traps fall into about 13 general categories with respect to construction material, size, and shape. Modifications on these general trap types with respect to number of tunnels, tunnel size, entrance size, and mesh size increase this number to greater than 30 different trap designs. The methods employed in fishing these traps also vary with respect to such things as: (1) soak time (usually from 3 to 96 h); (2) depth (between 15 and 250 m), and (3) baiting (at least six popular baiting methods).

This paper reports on a program to identify the key variables which should be accounted for in the definition of effective fishing effort for the British Columbia prawn fishery. Boutillier (1986) pointed out that catch-and-effort data analysis appears to be the only logistically feasible method for obtaining abundance estimates in this fishery at present. Only by developing an understanding of effective fishing effort will it be possible to develop and properly interpret catch-and-effort data to provide this stock assessment information.

MATERIALS AND METHODS

A series of three experiments were carried out in Alberni Inlet (Figure 1) during the summers of 1983 and 1984. Alberni Inlet is a deep (>300 m), steep, rocky, and rugged inlet on the west coast of Vancouver Island, which has historically been one of the major commerical prawn producing areas in British Columbia. The locations (Figure 1) were chosen from areas of typical rugged bottom between 55 and 110 m which had potential commercial quantities of prawns as determined by a series of prestudy sets. The experiments were designed to evaluate the necessity to include soak time, trap design, depth, tunnel entrance size, and their interactions into effort standardization algorithms such as those discussed by Miller (1983) for soak time. The variables measured in each of the three experiments are presented in Table 1.

In all of the experiments the captured prawns were removed from the study sites, counted, weighed, and sexed and their carapace length was measured. All traps were freshly rebaited after each haul.

In all three experiments, an analysis of variance for repeated

TABLE 1.

The variables tested in the three experiments designed to identify factors which need to be accounted for in the definition of effective fishing effort for the British Columbia prawn fishery.

	Variables			
	Soak Time	Trap Design	Depth	Entrance Size
1) Trap type (1983)	x	x	x	
2) Entrance size (1984)	x			x
3) Trap and entrance (1984)	x	x		x

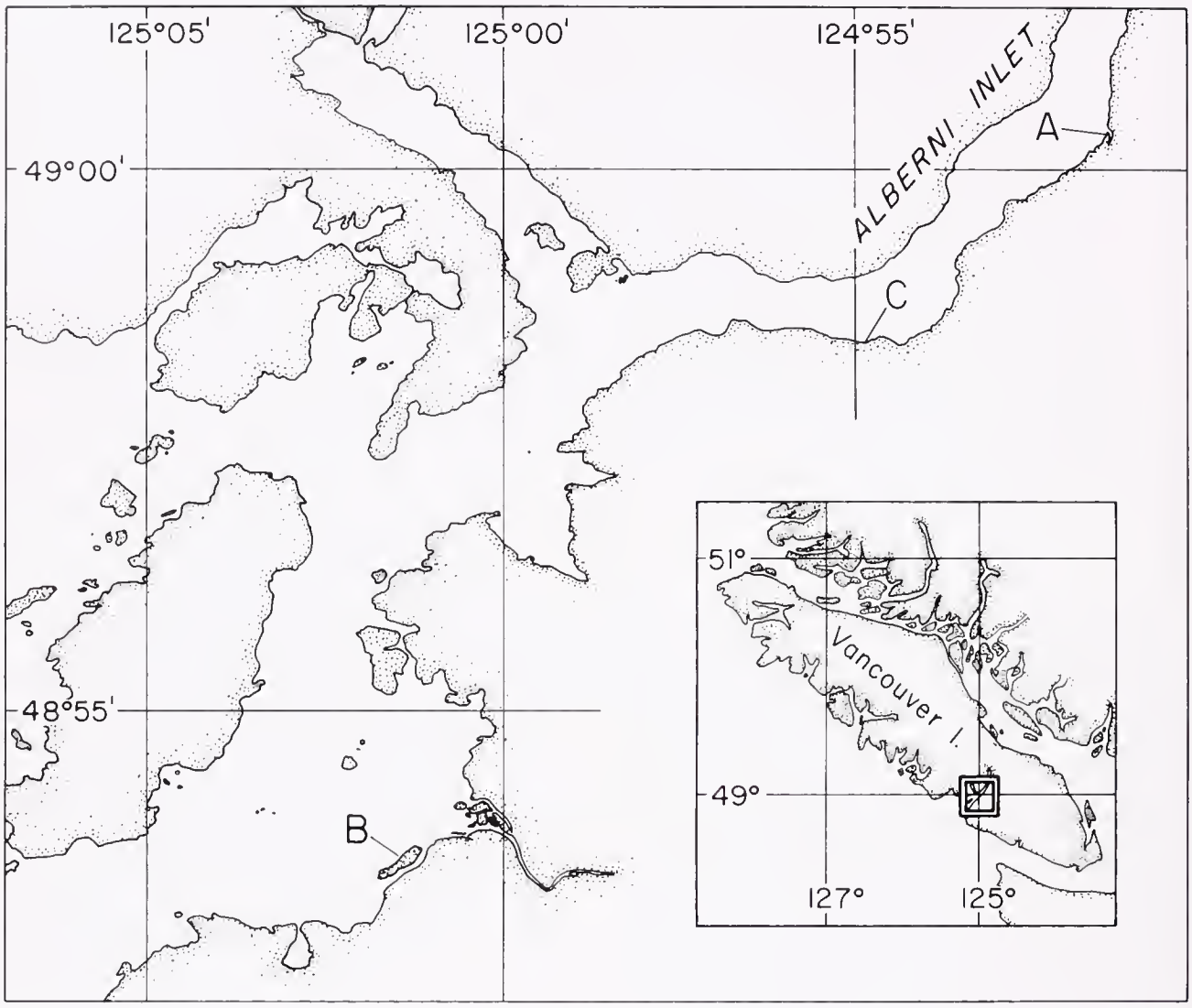

Figure 1. Alberni Inlet and the site locations of the 1983 and 1984 effort standardization experiments.

measure models with equal or unequal call size was carried out using programs from either the BMDP Biomedical Computer Program Package (Dixon and Brown 1979) or a SAS Institute statistical system (Freund and Littell 1981). The number of repetitions per experimental treatment may have differed when data from some traps were not used because the traps were lost, damaged, or pirated.

Bartlett's chi-square test for homogeneity of variance (Dixon and Brown 1979) was applied to all experimental data sets. In each case the variances were heteroscedastic ($P<0.05$) so that the data on catch per trap were transformed appropriately prior to conducting the analysis of variance.

Trap-Type Experiment (1983)

This experiment was designed to test soak time, trap design, depth, site, and their interactions. The experiment was carried out using seven types of popular commercial traps which are described in Table 2. Twenty-one traps, spaced at 18-m intervals, were fished on each groundline. The 21 traps consisted of three groupings of the seven trap designs, with traps set at random within any grouping. Data were obtained from groundlines that were fished along two depth ranges (55-75 m and 90-110 m) at each of two locations (A and B, Figure 1). All groundlines were randomly soaked for periods of 1, 2, or 3 days. Over the experimental period each soak time was repeated four times. This allowed for 10 to 12 repetitions for each of the 84 experimental cells.

Entrance-Size Experiment (1984)

This experiment was designed to test tunnel entrance size, soak times, and their interaction. For this experiment, a single string of gear was fished at a constant depth range (82-91 m) and location (site A, Figure 1). The string of gear consisted of 32 Pardiac type (Table 2) traps randomly fished at 18-m intervals along a groundline. This trap was selected because of the availability of research gear and because it is presently the

TABLE 2.

Codes, names, and description of the traps used in 1983 and 1984 fishing effort standardization experiments.

Code	Name	Shape	Size (cm)	Frame Material	Covering Material	Tunnel Entrances	Vol (*l*)
50	Pardiac	circular	59 diam. 25 high	aluminum	3.2 cm stretched web	3	58
52	Box	rectangular	33 wide 60 long 33 high	wood	plywood	2	52
55	Cone stackable	cone	66 top diam. 77 bottom diam. 31 high	steel	3.2 cm stretched web	3	113
56	Herring bucket	rectangular	27 wide 63 long 27 high	plastic	plastic	2	44
57	Rect. collapsible	rectangular	40 wide 54 long 33 high	steel	3.2 cm stretched web	2	61
60	Wire mesh	square	46 wide 46 long 23 high	steel	2.5x1.0 cm wire mesh	2	46
65	Butterfly collapsible	oval	48 wide 62 long 35 high	steel	3.2 cm stretched web	2	79

most commonly used trap in the British Columbia prawn fishery. Traps were modified with one of four different sizes of tunnel entrances (2.5, 5.1, 7.6, and 10.2 cm). Using a random schedule, the string of gear was fished twice at each of four soak times: 1, 2, 3, and 4 days. This allowed for a sample size of 16 repetitions for each of the 16 cells.

Trap and Entrance Experiment (1984)

This study was designed to test trap type, tunnel entrance size, soak time, and their interactions. For this experiment, a single string of gear was fished at a constant depth range (82-91 m) and location (site C, Figure 1). The string consisted of 40 traps randomly fished, at 18-m intervals along a groundline. The 40 traps included five replicates of four trap designs: the Box trap, Cone Stackable trap, Herring Bucket trap, and the Collapsible Butterfly trap (Table 2); each trap had one of two different tunnel entrance sizes (5.1 or 7.6 cm). These trap designs were selected to represent the most and least efficient of the traps as categorized by the 1983 experiment. The string of gear was fished seven times at each of two soak times: 1 and 2 days. This allowed for a sample size of 35 repetitions for each of 16 cells.

RESULTS

Trap Experiment (1983)

For a full factorial design, 3x2x2x7, of this experiment, neither the original data nor the log or square-root transformed data were found to be homoscedastic. After segregating the data by site, the square-root transformed data from both sites were found to have homogeneous variances. Treating the data at each site as a separate experiment was considered valid since they were two separate populations for which the processes acting upon the individual population were being tested.

In the two, three-factor analysis of variance with the square root of the (catch + 0.5) as the dependent variable, soak time, trap design, and a number of interactions were found to have a significant ($P<0.05$) effect on catch rates (Tables 3 and 4). Depth, however, was only found to significantly effect catch rates at site B (Table 4).

In an effort to try to understand more about the interactions, the mean catch for each trap type was plotted for each site versus soak time for two depth intervals (Figures 2 and 3). At Site A the mean catch per trap was only slightly higher in the shallow (55- to 75-m) sets (Figure 2A) than in the deeper (90- to 110-m) sets (Figure 2B) while at Site B the mean catch per trap in the

TABLE 3.

ANOVA results from the Trap experiment (1983) at Site A with square root of (catch + 0.5) as the dependent variable.

Source	Degrees of Freedom	Mean Square	F	Probability
Soak (S)	2	25.834	10.68	0.0000*
Depth (D)	1	8.350	3.45	0.0638
Trap (T)	6	27.158	11.23	0.0000*
S vs D	2	16.981	7.02	0.0010*
S vs T	12	3.894	1.61	0.0856
T vs T	6	1.692	0.70	0.6502
S vs D vs T	12	1.722	0.71	0.7404
Error	449	2.419		

*$P<0.05$

deep sets (Figure 3B) was greater than the shallow sets (Figure 3A). It was also found that trap type '55' (Cone stackable; Table 2) on average outfished all the other trap types.

A Tukey test, a multiple comparison procedure (Zar 1984), of the Site B interaction of depth and trap type found that the catch rates in the deep (90-110 m) for the Pardiac, Cone stackable, Rectangular collapsible, Wire mesh, and Butterfly collapsable were significantly ($P < 0.05$) greater than the catch rates for these same traps in the shallows (55-75 m). It also showed that for traps in the deep water the catch rate for the Cone stackable trap was significantly greater than all other trap types while there was no significant difference among any of the other trap types. In the shallow set the catch rate of the Cone stackable trap was only significantly greater than the Pardiac, Rectangular collapsible, and Wire mesh traps.

Entrance-Size Experiment (1984)

In a two-factor analysis of variance with the log of the (catch + 1.0) as the dependant variable, entrance size was found to significantly affect the catch rate (Table 5). The largest catches were taken in traps with 5.1-cm entrance openings, followed by 7.6-cm openings (Figures 4). In the extreme, the mean catch per trap between traps equipped with 2.5-cm and 5.1-cm openings differed by a factor of 5.9. A Tukey test of different entrance sizes found that the catch rates for 5.1-cm openings were significantly greater than for all other openings. The 7.6-cm opening had the second largest catch rates which were significantly greater than both the 2.5- and 10.2-cm openings. The difference between the catch rates for the 2.5- and 10.2-cm openings was not significant.

Trap and Entrance Experiment (1984)

In a three-factor analysis of variance with the log of the (catch + 1.0) as the dependant variable, trap type, entrance sizes, the soak-time/trap-type interaction, and trap type/entrance size interactions were found to be significant ($P < 0.05$) (Table 6). Table 7 shows the extent of variation between the mean catches of the significant interactions. As in the 1983 Trap experiment, trap '55' was the most efficient trap with catch rates greater than the least efficient trap '56' by a factor of 3. This difference increased to a factor of 3.6 when the soak time vs. trap interaction was accounted for and to a factor of 5.2 when the trap vs. entrance size interaction was taken into account. A Tukey test of the trap vs. entrance interaction found that the catch rates

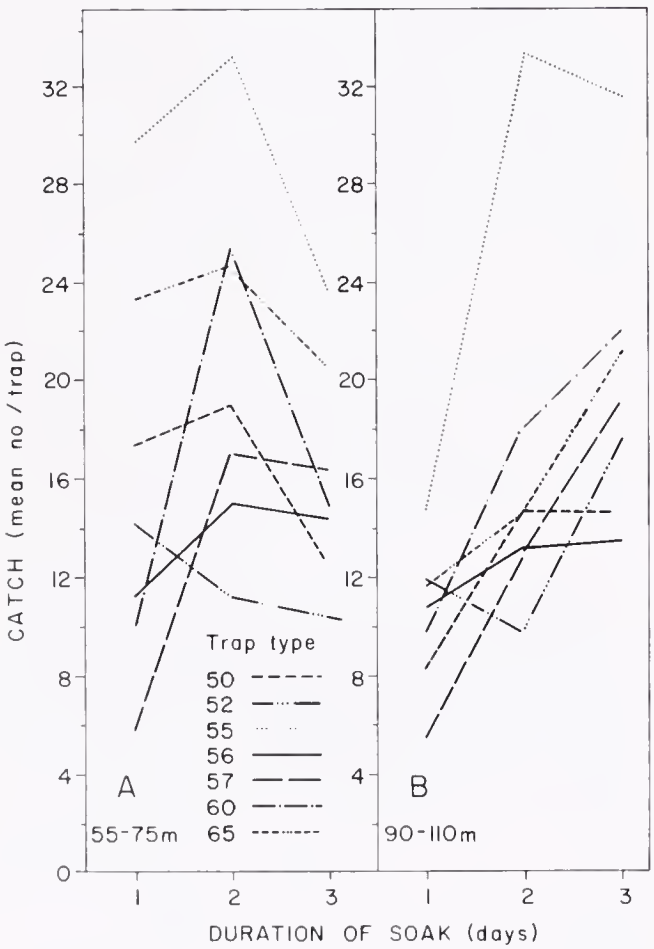

Figure 2. Mean number of prawns per trap of seven trap types at Site A, both depths, for the Trap experiment (1983).

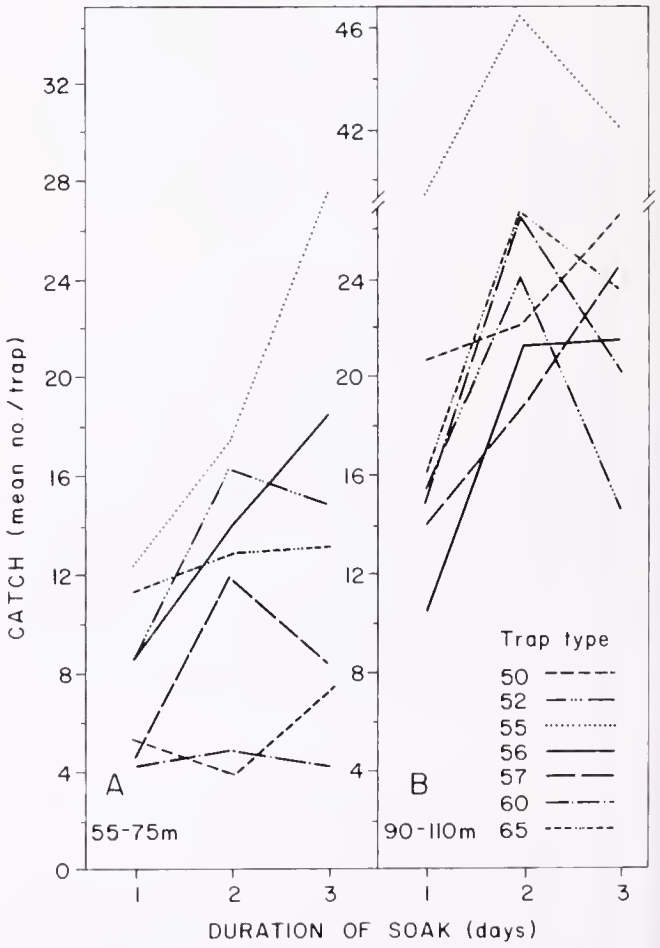

Figure 3. Mean number of prawns per trap of seven trap types at site B, both depths, for the Trap experiment (1983).

TABLE 4.

ANOVA results from the Trap experiment (1983) at Site B with square root (catch + 0.5) as the dependent variable.

Source	Degress of Freedom	Mean Square	F	Probability
Soak (S)	2	27.722	18.39	0.0000*
Depth (D)	1	335.954	222.91	0.0000*
Trap (T)	6	33.262	22.07	0.0000*
S vs D	2	3.158	2.10	0.1242
S vs T	12	2.011	1.33	0.1955
D vs T	6	10.752	7.13	0.0000*
S vs D vs T	12	1.923	1.28	0.2297
Error	452	1.507		

* $P < 0.05$

TABLE 5.

ANOVA results from the Entrance-size experiment (1984) with the log of (catch + 1.0) as the dependent variable.

Source	Degrees of Freedom	Mean Square	F	Probability
Total Catch				
Soak (S)	3	0.148	1.60	0.1899
Entrance (E)	3	4.904	52.90	0.0000*
S vs E	9	0.147	1.59	0.1202
Error	238	0.093		

*$P<0.05$

for the large opening Cone stackable ('55') trap was significantly ($P < 0.05$) greater than for all other traps except for the small opening Cone stackable and the large opening Butterfly collapsible ('65') traps. It was also found that for the Box ('52') and Herring bucket ('56') traps the 5.1-cm openings produced significantly higher catch rates than the same traps with 7.6-cm openings. A Tukey test of the trap vs. soak interaction found that for an individual trap type there was no significant difference between catch rates for the 1- and 2- day soak times. The significant differences occurred between trap types, with the catch rates from the Cone stackable trap ('55') for both soak times significantly greater than for both soak times from the Box ('52') and Herring bucket ('56') traps. The catch rates from the 2-day soak of the Cone stackable ('55') trap were also significantly greater than from the 1- day soak of the Butterfly collapsible ('65') trap.

DISCUSSION

Bennett (1974) and Bennett and Brown (1979) identified the difficulty in interpreting observed variations in catch-per-unit-effort (CPUE) from log-book data which do not provide information on the interactions of the animals attracted to the bait, the environment, and the trap. This series of experiments permit us to understand the actual complexity of interactions between the animals and the trap design which Simpson (1975) had reviewed in general terms. Treschev (1975) pointed out that in the use of catch-and-effort data for stock assessment there was usually no scientific basis for measuring the unit of effort. Our studies were designed to provide the scientific basis required to develop a catch-and-effort data base.

In the 1983 trap experiment, soak time, depth, and trap type were found to affect catches significantly; however, there were a number of interactions which confounded the interpretation of the relative significance of these main effects. Almost all of the significant interactions have depth interacting with the other main effects either individually or in combination. Boutillier (1986) found depth to be an important environmental variable influencing prawn catchability. Prawns are known to spend their first year of life in shallow waters (< 54 m), after which they emigrate into deeper (70-90 m) habitats (Butler 1980). Chew et al. (1974) and Boutillier (1986) both noted that prawns then undergo nocturnal migrations from these relatively high-density, deep-shelter areas to shallow-water forage areas. Through the use of the *PISCES*, a three man submersible vessel, Butler, Jamieson (Pacific Biological Station, Nanaimo, BC; pers. comm.), and the author (unpub. data) have all observed adult prawns during daylight dives in deep water. The prawns were associated in or near a narrow band of shelter-type habitat, i.e., under rocks and sunken trees, in holes and sponges, etc. At night, during scuba diving surveys, I found prawns actively foraging in open shallow water areas. Jamieson (pers. comm.) in night dives in the PISCES, also found prawns occupying a wider band of area extending into shallower water. This increased nocturnal activity and foraging behavior was also

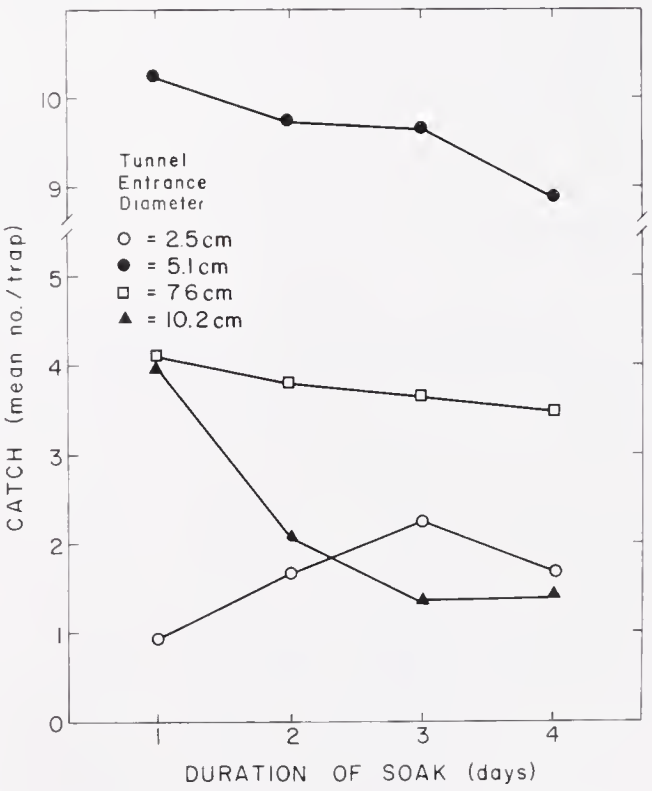

Figure 4. Mean number of prawns per trap of four entrance sizes for the Entrance-size experiment (1984).

documented by Barr (1967) for juvenile prawns and is well known in other Crustacea, such as the Norway lobster *Nephrops norvegicus* (Linnaeus) (Farmer 1975).

At Site A, similar catches from gear at both depth ranges would indicate that both sets were made in similar forage areas. At Site B, the large variation in catches between depths would indicate that the gear in the 55- to 75-m depth range was in a shelter area while the gear in the 90- to 110-m range was probably in a forage area. In graphing and *a posteriori* analysis of the interactions of traps, depth, and soak time, the one consistency which emerged was that the Cone stackable trap ('55') outfished all other traps. In speculating on the mechanisms causing this difference in fishing power of the various traps, the volume of the trap was a probable factor since the Cone stackable trap had the largest volume (Table 2). This, however, was probably not the only explanation and such factors as the shape of trap or the length of the tunnel will have to be evaluated to better understand the actual processes involved.

Depth selection was kept constant at each site for the 1984 experiments. Because the results from the 1983 trap study indicated that the shrimp exhibit preference with respect to depth. The gear was fished at depths of maximum concentration (i.e., sheltered habitat which was determined by conducting a pretest fishery). Since the shrimp undergo diel migrations to forage, the main concentration in the sheltered habitat can be assumed to be constant when the soak periods are multiples of the period of behavioural change (i.e., periods of 24 h). This eliminates the confounding interactions of depth with the other main effects.

Both the Entrance and Trap and Entrance experiments showed that entrance size had a significant effect on catch rates. In the Entrance experiment (Figure 4) and the Trap entrance experiment (Table 7) the 5.1-cm opening on average produced the highest mean catch rates. In the Entrance study, I found that the 2.5-cm openings were too small and impeded entry, while the 10.2-cm openings allowed entry but were so large that escape was very rapid. In addition to a high rate of escape, the 10.2-cm opening permitted the entrance of a large number of dungeness crabs (*Cancer magister* Dana) which were potential competitors and predators for prawns. In the Trap and entrance experiment, the trap type and entrance size interaction was interesting in that the most efficient traps ('55' and '65') had higher catch rates with the 7.6-cm openings while the less efficient traps ('52' and '56') had higher catch rates with 5.1-cm openings. A possible explanation is that the 7.6-cm entrances allow ready entry into all the traps but with the less efficient smaller traps this entrance size allows for greater escape with the longer soak periods than do the larger more efficient traps. A trap-type/soak-time interaction occurred in which the catch continued to increase in the more efficient Cone stackable ('55') and Butterfly collapsible ('65') traps with longer soak time, while the catch decreased in the less efficient Box ('52') and Herring bucket ('56') traps with longer soak times, presumably because the less efficient traps became saturated sooner and shrimp escaped overcompendated entry.

In an analysis of catch-and-effort data using an unstandardized unit of effort it would be impossible to differentiate the changes in stock sizes from changes in fishing effort patterns. For example a decline in CPUE from 500 g to 250 g per trap haul may mean that the population biomass has declined by 50% or that a vessel using Cone stackable ('55') traps fished the area first, followed by a vessel fishing Herring bucket ('56') traps. As the factors discussed above interact the synergistic variation becomes that much greater and the interpretation may be that much more misleading. Only through a process of evaluating these and some of the variables mentioned previously will it be possible to develop and properly interpret a catch-and-effort data base for stock assessment.

TABLE 6.

ANOVA result of the Trap and Entrance experiment (1984) with the log of (catch + 1.0) as the dependent variable.

Source	Degrees of Freedom	Mean Square	F	Probability
Soak (S)	1	0.158	0.92	0.3374
Trap (T)	3	4.648	27.09	0.0001*
Entrance (E)	1	0.975	5.67	0.0176*
S vs T	3	0.569	3.32	0.0195*
S vs E	1	0.573	3.34	0.0682
T vs E	3	1.525	8.88	0.0001*
S vs T vs E	3	0.091	0.53	0.6622
Error	543	0.171		

*$P<0.05$

TABLE 7.

The mean catch for interactions which were found to be significant in the Trap and Entrance experiment (1984).

Factor	Type	Mean Catch	Std. Error
Trap x Soak	52/1 day	7.61	0.752
	52/2 day	5.69	0.788
	55/1 day	14.99	1.662
	55/2 day	17.99	1.855
	56/1 day	6.20	0.650
	56/2 day	4.93	0.672
	65/1 day	9.02	1.054
	65/2 day	10.33	1.103
Trap x Entrance	52/5.1 cm	8.84	0.885
	52/7.6 cm	4.45	0.533
	55/5.1 cm	15.93	1.841
	55/7.6 cm	17.04	1.695
	56/5.1 cm	7.79	0.764
	56/7.6 cm	3.33	0.399
	65/5.1 cm	8.70	1.088
	65/7.6 cm	10.63	1.063

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THE EFFECT OF TRAP SOAK TIME ON YIELDS OF THE DEEP-WATER GOLDEN KING CRAB *LITHODES AEQUISPINA* BENEDICT IN A NORTHERN BRITISH COLUMBIA FJORD

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ABSTRACT In deep water ($\bar{X}$ = 320 m; range 185-402 m) fishing for fjord-dwelling golden king crabs (*Lithodes aequispina*) trap yields increased at a constant rate with soak time from 0 to 48 h. Between 48 and 96 h crab escape was significant. To delay trap saturation (reduction in catch rate with increasing catch), the possibility of curtailing crab escape by trap modifications is discussed. If crab escape can be curtailed, catch value can exceed the high cost of fishing these large, individually bouyed traps in deep water.

KEY WORDS: *Lithodes;* deep-water; soak time; trap yield

INTRODUCTION

A vital question for every crab trap fishermen is the value of added catch per trap with increased immersion (soak) time (Miller 1980). Yield based on trap soak time can also be useful to fisheries scientists as a component of stock assessments (Bennett and Brown 1979) and in adjusting catch per trap day to better reflect fishing effort (Austin 1977; Skud 1979). Despite the economic importance of the king crab trap fishery in the northeastern Pacific, little experimental work has been published on the effect of soak time on trap yield. Some data, however, are available from fishermen's log book analyses on crab escape from derelict traps by the shallow-water red king crab *Paralithodes camtschatica* (Tilesius) in Alaska (Rothschild et al. 1970; High and Worlund 1979).

Rapid development of golden king crab (*Lithodes aequispina* Benedict) resources in the deep waters of southeastern Alaska (T. Koeneman, Alaska Dep. Fish & Game, Petersburg, AK; pers. comm.) and the eastern Bering Sea/Aleutian Islands area (Otto et al. 1983) have occurred because of reallocation of fishing effort from the depressed stocks of red king crab (Armstrong 1983). Fishing costs are higher in deep-water trap fishing and may stimulate fishermen to alter their harvest strategies. Costs are increased due to poor weather at exposed sites in these northern latitudes, long trap-retrieval times, increased gear loss due to depth and exposure, and long search times for gear because buoys are submerged by tidal currents (T. Koeneman; pers. comm.).

We report on deep-water trap yields of *L. aequispina* according to different soak times in Alice Arm, a fjord in northern British Columbia. Minor commercial landings (<4300 crabs) were taken between 1980 and 1982 (Jamieson and Sloan 1985). The experiment adheres to Miller's (1983a) criteria for trap yield studies by establishing an area of similar catch (*uniformity trial*) and maximizing uniformity of key variables such as fishing technique, equipment, and baiting, while manipulating one major variable (soak time) and injecting some element of randomness (trap sites within the fjord).

MATERIALS AND METHODS

Alice Arm (55°28'N, 129°36'W) is a deep fjord comprising an extremity 95 km inland of the mouth of the Portland Inlet system, northern British Columbia. It is a basin (maximum depth ≈ 402 m) isolated by a sill 18 m deep near its mouth (Pickard 1961). The trough area (>183 m depth) of Alice Arm covers ≈ 7.0 km², is 13.3 km long and has a maximum width of 1.2 km.

In October, 1983 a *uniformity trial* consisting of 44 nonrandom, individually buoyed traps were deployed throughout the trough of Alice Arm. After establishing the presence of the deep-water target species *Lithodes aequispina* throughout the trough, two subsequent samplings occurred in March and July, 1984. In March, 19 to 20 traps were deployed at coordinates generated by random number tables within the trough with soak times of 24, 48, and 96 h. In July, 19 to 21 traps were similarly deployed within soak times of 6, 12, and 24 h. Crabs' carapaces were scratched, to signify capture, and they were released while as the vessel steamed between traps. Less than 6.0% of the catch was retained. Any mortalities in the traps were noted. Standardization of trap setting time was approximated by deploying traps between 0700 and 1300 h. This was probably not essential because the crabs live in uniformly poor light conditions at great depths in the narrow, steep-walled fjord.

Alaskan, side-entry, king-crab traps measuring 1.8 x 1.8 x 0.9 m with 9.0 x 12.0 cm polypropelene mesh were used. Each trap had two side tunnels with 18.5 x 89 cm (internal dimension) "tunnel eyes." Traps were always baited with a pair of 2-*l* perforated (2.0-mm diameter holes) jars of chopped frozen herring from the same supplier throughout the study. Each jar contained a mean weight of 0.8 kg of bait (range 0.75 to 1.05 kg; n = 55).

RESULTS

Table 1 lists the depth, yield, and catch rate per 24 h for *Lithodes aequispina* according to different soak times. Catch

Table 1.

Catches of *Lithodes aequispina* according to different trap soak times in March and July, 1984, in Alice Arm, British Columbia.

Soak times (h)		No. of	Trap depths (m)		*L. aequispina* per trap		
					Yield per trap		Catch rate** (crabs d^{-1})
Mean $\bar{X}$	Range	Traps	Mean* $\bar{X}$	Range	$\bar{X} \pm SD$	Range	$\bar{X} \pm SD$
			March, 1984				
95.4	93.0-96.8	19	326	210-399	17.7±13.1	1-63	4.6±3.1
44.7	42.7-48.4	20	325	229-390	26.6±11.3	7-47	14.2±6.0
24.1	22.2-25.7	20	313	187-402	14.9±8.0	2-31	14.9±7.9
			July, 1984				
23.8	22.0-25.3	20	309	199-384	11.9±7.9	4-40	12.3±7.6
13.3	12.8-13.8	19	339	329-399	8.2±4.1	1-17	14.9±7.4
6.2	5.5-7.0	21	312	185-397	4.5±4.4	0-16	17.7±17.8

*Mean for all trap depths = 320 m.
**Calculated from the hourly catch rate X 24.

increased in direct proportion to soak time from 6 through 48 h, then decreased at 96 h (Table 1; Fig. 1). Analysis of variance demonstrated that the catches of the 24-h soak times in March and July were not significantly different ($P > 0.05$). The 24-h trap in July which yielded 40 crabs had a by-catch of two large Pacific cod (*Gadus macrocephalus* Tilesius) which the crabs were feeding upon. With this trap excluded from the July data, the mean catch for the remaining 19 traps was 10.4 (±4.2 S.D.), which was significantly lower than the March catch ($p < 0.05$). Analysis of variance revealed that the daily catch rate of crabs for the 96-h trap was significantly lower ($p < 0.05$) than all other soak times. The combined daily catch rates from 6 h through 48 h soaks from both samples were not significantly different ($p > 0.05$). Three individuals were recaptured. Four crabs died in traps that were soaked 48 h and three in traps soaked 96 h.

DISCUSSION

Deep-water trap fishing for some *Lithodes* spp. has been suggested as uneconomical in subantarctic (Arnaud et Do-Chi 1977) and Gulf of Alaska waters (Somerton 1981). Problems were low catch rates, difficult fishing conditions due to depth (to 800 m), and poor weather conditions. On the other hand, southeastern Alaskan fishermen have been attracted to the high landed-value of *L. aequispina* despite the relatively high cost of fishing in deep-water. In years of low effort and long seasons, soak times of up to 14 days were common (T. Koenenman; pers. comm.). The industry standard of approximately 48 h soak times for shallow-water, red king crab (*Paralithodes camtschatica*) traps has been decreased to <24 h during conditions of short, intense seasons and high, exvessel value (T. Koeneman; pers. comm.).

In our experiment the *saturation effect* (Miller 1979) of decreased catch per trap-day with increasing soak time did not occur up to 48 h, but changed dramatically at some time between 48 and 96 h. This resulted at least partly from crab escape; however reduced entry, perhaps related to reduced herring bait quality over time (High and Worlund 1979), could not be ruled out as another contributory factor. Catch rate rarely remains

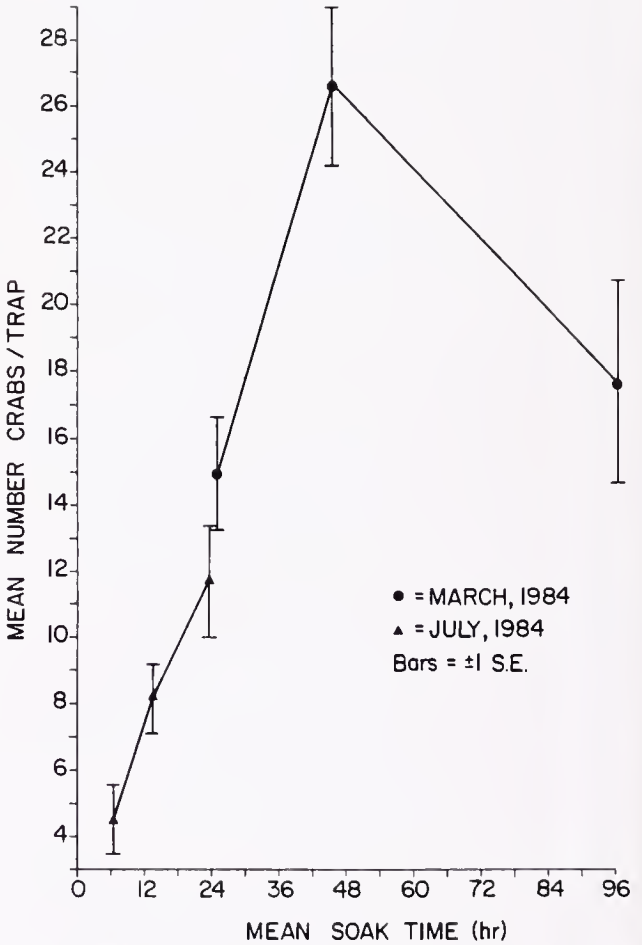

Figure 1. Mean number of *Lithodes aequispina* (±1S.E.) per trap for soak times between 6 and 96 h. (S.E. = 1 Standard Error of the mean.)

as nearly constant as we observed over 48 h (R. J. Miller, Dept. Fisheries and Oceans Halifax, N.S., Canada, pers. comm., 1983a, 1983b). The entry rate of *P. camtschatica* into baited traps peaked at 3 days as did escape, which reached >80% of the total catch (High and Worlund 1979). Log-book data have shown that deep-water lobster yields can be improved over long (48 to 72 h) soaks (Skud 1979). Among *Cancer* spp. for which much smaller traps are used, saturation effects can appear within 4 h (Miller 1979, 1980) and crabs readily escape (Muir et al. 1984).

Plastic collars in the tunnel eyes of Alaskan king crab traps, which were not installed in our traps, appreciably decrease the escape of *L. aequispina* (T. Koeneman; pers. comm.) as they do for other crab species (Miller 1980). High and Worlund (1979) used side-entry king crab traps with two similar-sized tunnel eyes to our study (without collars), and recorded high levels of escape of *P. camtschatica*. We expect that the addition of collars would increase the catches of *L. aequispina* for soak times longer than 48 h.

The relatively high variance of the catch rate for 6-h soak times may have resulted from the fortuitous landing of traps near patches of *L. aequispina*, thus permitting high catch rates versus traps that landed in areas without groups of crabs. The lower and more uniform variance of the catch rates for longer soak times suggests a more regular movement of crabs towards traps over time, until crab escape becomes significant, after the effects of initial trap settlement on the bottom.

Our results indicated no seasonal variation in vulnerability of these deep-water (>300m), fjord-dwelling golden king crabs to entrapment. This may be an artifact, however, as a single 24-h trap in July yielded an unusually high catch (40), possibly because of the two large fish by-catch carcasses being torn apart by the crabs. Without this trap the mean July catch (for 24-h traps) was significantly less than March catches and similar to catches from traps at all deths described by Jamieson and Sloan (1985). Seasonal vulnerability could nonetheless be dampened in *L. aequispina* as it did not demonstrate a seasonal reproductive cycle in the fjords (Sloan 1985). Shallow-water lobsters and crabs can demonstrate marked seasonal vulnerability to trapping (Bennett 1974; Morgan 1979; Rodhouse 1984) as does the American lobster *Homarus americanus* Milne-Edwards which is fished in both shallow- and deep-waters (400 m) (Skud 1979).

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RESEARCH NOTE

CULTURE OF THE SHRIMP *PENAEUS VANNAMEI* BOONE USING FEED AND TREATED WASTEWATER

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ABSTRACT The production of shrimp from three ponds, on different feeding regimes, was compared. One pond received a commercial feed, another only secondarily treated wastewater, and the third was started on wastewater then switched to feed. By switching from wastewater to the prepared diet, a good yield was obtained yet the cost of feeding was reduced.

KEY WORDS: *Penaeus vannamei*, shrimp, aquaculture, wastewater

INTRODUCTION

Perhaps the most important cost factor associated with the culture of marine organisms is the feed. Ryther et al. (1972) suggested that this can be ameliorated by the addition of treated wastewater into aquaculture systems. The nutrients in the wastewater will support primary and secondary production, which in turn support the animals being cultured; however, shrimp of the genus *Penaeus* have not responded well to this treatment (Ryther, unpublished). While postlarvae of *P. stylirostris* Stimpson, *P. duorarum* Burkenroad, and *P. setiferus* (Linnaeus) grow well initially under such conditions, growth is severely curtailed when the shrimp reach the 3- to 6-gram juvenile stage (Ryther, Williams, and Landau, unpublished). The hypothesis tested here was that *Penaeus vannamei* Boone could be grown efficiently by using only wastewater until they reached 3-6 grams, followed by a prepared commercial feed for the duration of their growout.

MATERIALS AND METHODS

Three 50,000-*l* earthen ponds (10 x 10 x 0.5 m) lined with 6-mil, PVC plastic sheets were stocked on 15 May 1982 with 19 to 23-mg postlarvae of *P. vannamei* at an initial density of 2400 per pond. Two weeks before the postlarvae were added, the ponds were fertilized with ammonium nitrate and sodium phosphate to stimulate primary production (10 mg $N \cdot l^{-1}$ and 1 mg $P \cdot l^{-1}$); this inorganic fertilization continued once every 3 to 4 days until 16 June. All of the ponds received water from the Harbor Branch Foundation ship canal, an extension of the east central Florida Indian River Estuary system. The water was pumped into the ponds at a rate of 12,500 $l \cdot day^{-1}$. The water conditions varied with the local climate, which included high rainfall during the mid- and late-summer; salinities varied from 16.3 to 38.1 $^{0}/_{00}$, and the temperature varied from 24° to 33°C. The shrimp were harvested 210 days after stocking on 11 December and final counts and weights were determined. Ponds were also sampled every 2 weeks during the experimental growout period to monitor shrimp growth and survival, using 3 or 4 unbaited minnow traps placed along the edge of each pond over night; these shrimp were returned to the ponds after weighing.

Shrimp in one of the ponds (I) were fed a pelleted diet, Ralston Purina Experimental Marine Ration LF (25% protein, 5% crude fat, 5% crude fibre). Shrimp received approximately 10% of their wet-weight per day for the first 2 months (20-100 $g \cdot day^{-1}$). Thereafter, the absolute amount of feed was maintained (100 g) until the resulting feed: wet-weight ratio had decreased to 2-3% $\cdot day^{-1}$ (i.e., when shrimp reached approximately 2 g); at that point, the amount of feed was increased every 2 weeks so that the 2-3% ratio was maintained for the duration of the experiment (4 mo). Feeding rate calculations were based on a 100% survival. Pond II received a mixture of treated wastewater and water from the ship canal (1:9, wastewater:seawater) for the first 89 days of the project; at that point the pipe bringing in the wastewater was closed and the flow of canal water was increased by 10%. After the wastewater treatment was halted, the shrimp were switched to the pelleted feed for the last 121 days. The mean individual weight of the shrimp in pond II was 2.55 g after 89 days. The wastewater from the secondary, activated-sludge, treatment plant was comparatively low in nutrients in contrast to municipal treatment plants (0.03 mg N as $NO_3 + NO_2 \cdot l^{-1}$; 0.37 mg N as $NH_4 \cdot l^{-1}$; 1.3 mg P as $PO_4 \cdot l^{-1}$;) (Landau 1983). Pond III received the same mixture of the canal water and wastewater effluent as pond II; that mixture was

maintained for the entire experimental period.

RESULTS AND DISCUSSION

The growth data for the shrimp are summarized in Table 1. The final yield for pond III, which received only the diluted wastewater, was considerably less than either pond I or II. This may be a result of: (1) an inadequate diet, in terms of either the amount of material available or the quality of the material that the shrimp fed on; or, (2) substances which could have been present in the sewage effluent such as heavy metals or chlorinated organics (Landau 1983).

Penaeus vannamei grew much more quickly than *P. stylirostris* under similar conditions (Landau et al. 1985), and yields of over 1,000 kg·ha^{-1} may make it a viable candidate for commercial culture. Anderson and Tabb (1971) modelled the economics of operating shrimp farms in Florida that were designed for the production of adult shrimp for human comsumption and for bait shrimp. They found that for 1,000-ac food and bait farms, the costs of feed constituted 27.8 and 41.3% of the annual expenses, respectively. If feed costs could be excluded during the first three months of the food-shrimp farm's operation, a substantial economic gain would, of course, result. For the bait farm, virtually the entire 41.3% of the annual costs could be eliminated because the growout period is only 3 mo per crop; the use of treated wastewater would mean a profit increase and would ease the cash-flow problems which beset so many aquaculture operations.

TABLE 1.
Survival, mean weight, and projected yield from the three shrimp ponds after 210 days.

Pond	Treatment	% Survival	Weight (g) mean ± S.D.	Projected Yield (kg·ha^{-1})
I	Feed	90.5	6.3 ± 3.0	1,370
II	Effluent + Feed	72.5	9.4 ± 2.1	1,640
III	Effluent	17.6	5.0 ± 0.5	210

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ABSTRACTS OF TECHNICAL PAPERS

Presented at the 1984 Annual Meeting

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CHANGES IN POPULATION STRUCTURE FROM 1979 TO 1983 RELATED TO INCREASED SPAT SETTING ON AN OYSTER BAR IN CENTRAL CHESAPEAKE BAY

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Oyster densities on the 275-ha (680-a) Flag Pond Oyster Bar near the Calvert Cliffs Nuclear Power Plant in central Chesapeake Bay were 1.4, 0.6, and 0.1·m^{-2} for legal oysters ($\geq$76 mm), sublegals (25-75 mm), and spat (<25 mm), respectively, in 1979 because of poor recruitment during the previous decade. Increased spat setting throughout much of the bay in 1980-82 resulted in new studies of the bar in spring and fall 1983 to document any changes. Seven areas were sampled at 169 locations by divers who removed all oyster components (shells, boxes, live oysters) from within three 0.33-m^2 replicates at two positions at each location. A total of 1010 samples yielded 1064 legal oysters (3.2·m^{-2}), 6889 sublegals (20.5·m^{-2}), and 203 spat (1.6·m^{-2}). Of the 7 areas examined, oyster densities were moderate in 4 and low in 3. Overall densities in 1983 were 2.3 times the 1979 levels for legal oysters and 34 times the 1979 levels for sublegals. Spat density was also higher, but apparently nowhere near the 1980-82 levels. Analysis of the data using a nested analysis of variance revealed for most components in spring that variance among areas > variance among locations > variance between positions > variance among replicates ($p<0.01$). In the fall the variances among areas and among locations did not differ significantly; otherwise the results were similar to spring. Present population size of Flag Pond Bar is estimated at 54,000 bu (1902 m^3) of oysters, nearly 8 times the 7000-bu (247-m^3) estimated in 1979. This estimate should increase during the next 2 yr as sublegal oysters enter the legal size class.

ARKS—IS THERE A RESOURCE AND A MARKET?

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Making the initial investment to identify and develop a market for a marine animal not consumed locally is often beyond the scope of most commercial fishing interests. Uncertainties such as the extent of the resource, susceptability to commercial harvesting, and marketability are obstacles to introducing a morphologically similar species into foreign and domestic seafood markets.

Three red-blooded pelecypods, the blood ark *Anadara ovalis* (Bruguière), the incongruous ark *A. brasiliana* (Lamarck), and the ponderous ark *Noetia ponderosa* (Say), which are similar to commercially exploited bivalves in Europe and Asia, were selected from South Carolina estuarine wild stocks for possible commercial harvesting and marketing. Concurrent with hydraulic escalator harvesting of *Mercenaria mercenaria* (Linné), the arks are caught incidentally in certain estuarine and offshore waters in the South Atlantic bight. During the summer of 1983, 6 estuaries and 4 offshore areas were assessed with a 12.8-m, hydraulic escalator dredge. The 3 species were processed by measuring shell dimensions, separately weighing meats and valves, and sealing the meat product in 500-g plastic bags. Objectives of the project consisted of locating commercial concentrations of the resource and developing information on potential foreign and domestic markets. Questionnaires packaged with valves of each species were forwarded to selected seafood companies in 10 countries followed by shipment of live samples to interested businesses in the United States.

THE EFFECTS OF PREY SIZE, PREDATOR SIZE, AND SEDIMENT COMPOSITION ON THE RATE OF PREDATION OF THE BLUE CRAB *CALLINECTES SAPIDUS* RATHBUN ON THE HARD CLAM *MERCENARIA MERCENARIA* (LINNÉ)

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Sediment preferences of blue crabs and predation rates on various size-classes of hard clams in a variety of sediment types were studied in the laboratory. All blue crab size-classes exhibited a preference for sand, mud, and sand/mud sediments rather than crushed oyster shell or granite gravel. Sediment feeding tests indicated that clams were significantly more vulnerable to predation by crabs in sand and sand/mud than in crushed oyster shell or granite gravel, although the outcome of such predatory encounters depend upon the interaction between clam and crab size. When crabs were given a choice of clam sizes, with no sediment present, small crabs (< 75 mm carapace width [CW]) consumed 5- and 10-mm size-class clams equally. Medium crabs (75-125 mm CW) preferentially consumed 10-mm size-class clams. Large crabs (> 125 mm CW) consumed 10- and 25-mm size-class clams equally. Clams larger than 40 mm CW were not consumed by even the largest blue crabs, indicating that hard clams may achieve a size refuge from blue crab predation.

THE EFFECTS OF LIMB REMOVAL ON MOLTING IN THE BLUE CRAB *CALLINECTES SAPIDUS* RATHBUN

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Limb removal was studied as a possible means of inducing and synchronizing ecdysis in juvenile blue crabs that were held in closed, recirculating seawater systems. Cheliped removal in intermolt crabs (40-100 mm in size) did not significantly shorten the time to molt, but did reduce the variance of this time when compared to intact controls. The removal of both chelipeds and four walking legs in crabs (100-140 mm), however, shortened the time to ecdysis and reduced the variance of this time to molt when compared to chelotomized crabs of the same size range. Comparison of the percent size increase in carapace width following ecdysis revealed a significant difference between controls and crabs with chelipeds removed and multiple appendages removed (24.0% vs. 18.0% and 19.4%). In chelotomized crabs, the average cheliped length following ecdysis was 96.7% of the pre-autotomy cheliped length. Multiple-limb autotomized crabs regenerated chelipeds 94.2% of their preautotomy length. The relationship between limb regeneration and molt stage was examined. Regeneration indices were calculated on the developing chelipeds of both chelotomized and multiple-limb autotomized crabs and proved useful in predicting time to molt. Morphological changes of the developing limbs were also found to be indicators of the time to ecdysis.

$O:NH_3$ AND $CO_2:O_2$ (RQ) RATIOS AS INDICATORS OF CONDITION IN THE BAY SCALLOP *ARGOPECTEN IRRADIANS CONCENTRICUS* (SAY)

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Molar ratios of oxygen consumption of ammonia excretion ($O:NH_3$) and carbon dioxide production to oxygen consumption (RQ) for the bay scallop were found to fluctuate seasonally in response to reproductive development and the resulting shifts in energy metabolism. Resting stage animals (May-early June) with small meat (adductor muscle) weight ($\overline{X}$ =0.6 g dw) and low glycogen level (11%) had a mean $O:NH_3$ ratio of 12.0 and a mean RQ of 0.7. Scallops in the initial stages of gametogenesis (late June-early August) with maximum meat weight ($\overline{X}$ =2.1 g) and glycogen level (28%) had a mean $O:NH_3$ ratio of 22.0 and a mean RQ of 1.6. As gamete development continued (late August-September), meat weight and glycogen level were drastically reduced as were the $O:NH_3$ and RQ values. Postspawn scallops (October-November) were characterized by decreased meat weight ($\overline{X}$ =0.7 g) with minimum glycogen level (3%) and a mean $O:NH_3$ ratio of 9.0 and a mean RQ of 0.6. Bay scallops in Florida were found to undergo a yearly growth cycle in which maximum meat yield (50-75 count) with maximum glycogen content occurred in August in conjunction with maximum $O:NH_3$ and RQ values. We concluded that $O:NH_3$ and RQ ratios were sensitive, nondestructive indicators of bivalve condition (meat weight and carbohydrate content).

POPULATIONS OF *MERCENARIA MERCENARIA TEXANA* (GMELIN) IN TEXAS BAYS AND THEIR COMMERCIAL POTENTIAL

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Moderate to low, contagious populations of Texas quahog clams occupy high-salinity portions of Texas bays intertidally to over 3-m depths (rarely over 4 clams·m^{-2}, usually much less). Small clams, less than 3 yr old, predominate directly adjacent to major passes, indicating more favorable conditions for recruitment and early survival. With increasing distance from the passes, population size-frequency composition shifts toward larger and older clams (upper Christmas Bay contains mostly clams 4 yr and older). Growth rates are comparable to those of the northern quahog clam *Mercenaria mercenaria* (Linné) in Florida (30-40 mm high in first year). Within Texas, growth appears somewhat more rapid in the southern bays (Corpus Christi) than in the northern bays (Galveston). Natural populations will not support a clam fishery in Texas, but hatchery development and a bay seeding program may support such a fishery.

NUTRITIONAL REQUIREMENTS FOR TOXIN PRODUCTION BY A PATHOGENIC *VIBRIO* SPECIES

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A *Vibrio* species isolated from hatchery-reared and diseased larvae of the American oyster *Crassostrea virginica* (Gmelin) was shown to produce a teratogenic metabolite when grown in a complex medium. Characterization of this metabolite revealed that it is a heat-labile, proteinaceous exotoxin, having a molecular weight of about 68,000. Nutritional study of the *Vibrio* sp. determined that the pathogen requires several inorganic salts, glucose, and asparaqine for growth. Bioassays demonstrated that toxin is not produced in this chemically-defined medium. Toxin production, however, does occur if the medium is supplemented with hypoxanthine and either glutamic acid, histidine, or sodium thiosulphate. Characteristics of the exotoxin produced in the synthetic medium are compared with those of exotoxin produced in the complex medium.

FARMING OF THE NORTHERN HARD CLAM *MERCENARIA MERCENARIA* (LINNÉ) IN VIRGINIA

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Clam farming has been practiced in Virginia since the 1920's. Historically, clam shippers would relay harvested clams onto intertidal flats so they could be reharvested when the market demand and price were more favorable. On the Eastern Shore of Virginia from the 1930's to early 1950's, several leaseholders purchased "buttons" (larger sized natural seed about 10 to 20 mm length) which were planted on intertidal or shallow subtidal areas and later harvested when they had grown to market size. Clam farming that uses hatchery-reared seed is a relatively new industry in Virginia. Currently about 12 individuals or companies are farming clams, of which three have been in operation for more than 5 yr. Most of this seed was purchased from commercial hatcheries in New York or Massachusetts. Four of the Virginia companies have their own hatcheries and nurseries with the potential of producing excess seed to sell. Clam seed are grown to littleneck size using a variety of field grow-out methods. Clams are grown in trays, under nets, under nets with gravel or shell aggregate, or by a combination of these methods. Market size of approximately 25 mm width is reached in 2 - 3 yr.

A REVIEW OF WEST COAST OYSTER FISHERIES AND THE PRESENT IMPACT OF REMOTE SETTING OF OYSTER SEED

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A brief account is given on the trends in the oyster fisheries. The present fishery centers almost exclusively around the fresh market. In recent years, the demand for oysters on the east coast of the United States has had some impact on production in the Pacific Northwest. The demands from both the east and west coasts have, in part, created a situation in which oysters may be harvested at an earlier age to accomodate the market. To complicate the situation further, the recent El Niño may have caused significant setbacks in the harvest of Pacific oysters from coastal bays such as Willapa Bay in Washington State, historically one of the most productive bays on the west coast. Problems and situations related to this climatic phenomenon will be discussed. Remote setting of eyed larvae for the west coast is a reality and adjustments by many of the oyster growers are being made to accomodate this latest technique for securing Pacific oyster seed. The potential of remote setting has essentially taken away most of the guess work related to securing adequate seed for growing in most of the areas along the Pacific coast of the United States.

DEVELOPMENT OF TECHNIQUES TO STUDY ACQUIRED IMMUNITY TO *PERKINSUS MARINUS* (MACKIN, OWEN & COLLIER) IN THE AMERICAN OYSTER *CRASSOSTREA VIRGINICA* (GMELIN)

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Attempts have been made to develop techniques to study phagocytosis of zoospores of *Perkinsus marinus* by hemocytes from oysters previously immunized with *P. marinus* zoospores. Three culture media have been tested for their capability to maintain oyster hemocytes in a viable state in order to study their phagocytic response to *P. marinus* zoospores. The three media were: 1) minimum essential medium (MEM), 2) lobster hemolymph medium (LHM), and 3) NCTC-135 medium. All three media were found to maintain oyster hemocytes in a viable state at 10, 15, and 25°C for up to 26 h, although the survival rate of hemocytes cultured in LHM was slightly lower than for those cultured in either of the other media. We have successfully radiolabeled zoospores of *P. marinus* with uniformly labeled ^{14}C-glycine. This is necessary for

studies to quantify phagocytic activity. Leaching of radioactivity from zoospores ranged from 0 to 25% after 24 h in label-free medium. A technique using a Percoll gradient of 1.04 to 1.055 $g \cdot ml^{-1}$ has been developed to separate nonphagocytized zoospores from hemocytes. Currently we are measuring the efficiency and reliability of this technique.

NUTRIENT PROCESSING BY OYSTER REEFS

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Oyster reefs are dense concentrations of filter-feeding consumers. A flow-through plastic tunnel, benthic ecosystem tunnel (BEST) was shown to be a reliable and portable method for determining significant changes in material concentrations in tidal water passing over an oyster reef. Significant reductions were found in particulate organic carbon and chlorophyll *a* concentrations, while ammonia concentrations were significantly increased. Utilizing water velocities concurrently measured with water samples from BEST, estimates of ammonia release rates were very high during summer conditions, 1682 to 7253 μg at.$\cdot m^{-1} \cdot h^{-1}$. Nutrient fluxes of the magnitude reported here support the idea that oyster reefs play an important role in material cycles in marsh-estuarine ecosystems.

PRELIMINARY OBSERVATIONS OF THE *BUSYCON* WHELK FISHERY OF VIRGINIA

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The knobbed whelk *Busycon carica* (Gmelin) and the channeled whelk *Busycon canaliculatum* (Linné) constitute the whelk fishery of Virginia. *Busycon carica* is harvested primarily in a direct summer dredge fishery and as by-catch from crab dredging. Peak landings occurred in 1966 with 215,900 kg (476,000 lb) with a value of $70,500. *Busycon canaliculatum* is harvested primarily as by-catch from surf-clam, crab-pot, and flounder-trawl fisheries. Peak landings occurred in 1974 and 1975 with 549,400 kg (1,025,000 lb) and a value of $110,000 for each year, coincident with peak surf-clam landings. Landings for 1982 for both species totaled nearly 57,650 kg (127,100 lb) with a value of $83,000. Whelks are a fishery resource with great potential for exploitation. This research was undertaken to describe the Virginia Chesapeake Bay and offshore fisheries in terms of population parameters. Length, width, weight, sex, and species frequencies have been determined from monthly samples from Virginia commercial landings from July 1983 to present. *Busycon carica* ranges from 75 to 250 cm length, 40 to 120 cm width, and 50 to 550 g weight. *Busycon canaliculatum* ranges from 80 to 200 cm length, 40 to 110 cm width, and 65 to 370 g weight. For both species females predominate over males, particularly in larger size classes. While most reports describe whelks as "slow-growing," mark-recapture data have indicated the possibility of rapid growth, with as much as 9.7 cm of shell laid down at the aperture over 2.75 yr. Total increases of 18.8 cm length, 8.2 cm width, and 101.1 g weight were determined for females of *Busycon carica*. Increases of 3.7 cm length, 1.5 cm width, and 30.6 g total weight were determined for males of *Busycon carica*. These estimates were drawn from samples collected from July 1983 to March 1984.

SUCCESSFUL USE OF CRAB MEAL AS A SUPPLEMENTAL FOOD FOR JUVENILES OF THE HARD CLAM *MERCENARIA MERCENARIA* (LINNÉ)

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Nursery culture of the hard clam, a necessary step in the production of seed for field growout, is not considered economically feasible by many workers. This results primarily from costs involved in supplying the large quantities of food required by juvenile clams. An inexpensive supplemental food could greatly alleviate feeding costs. Commerically available crab meal, a byproduct of crab picking houses, was tested as a supplemental food with various sizes of juvenile hard clams. Six, 30-day feeding experiments were conducted from July to December 1983. In each experiment, both control and crab-meal fed groups received filtered seawater at flow rates which contained enough natural food to support clam maintenance activities. The test groups also received crab-meal supplements at different rations proportional to the total live weight of the clams. Growth was evaluated as the increase in shell height, and total live, dry, and ash weights. In all experiments, significantly greater increases in shell height and weight were seen in supplemented clams compared to control clams when crab meal was fed in proper amounts. Optimum feeding rates for smaller clams

(4-6 mm) were crab meal rations of 20 to 25% of total clam live weight per day, and for larger clams (7-10 mm) rations were 10 to 12% of clam live weight per day. Overall, crab-meal fed clams showed increases in weight and shell height from 10 to 100% greater than in control clams.

THE CORTICAL RENAL EPITHELIA OF THE HARD CLAM *MERCENARIA MERCENARIA* (LINNE)

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The kidney of the hard clam is situated in the middorsal region in the vicinity of the hinge ligament and just anteriad the heart. Because of its unique location, the kidney has two different types of cortical epithelia: the shell-side epithelium is in juxtaposition to the shell and closely resembles the shell-side mantle epithelium with which it is contiguous; the mantle-cavity epithelium faces the pallial cavity and is partially covered with a portion of the gills. The shell-side cortical epithelium is simple columnar with basally situated nuclei; the cytoplasm is faintly eosinophilic and contains many bundles of microfilament of microtubule-like structures oriented in the long axis of the cells. These bundles originate on the basal lamina, traverse the entire length of the cell, and terminate on the plasma membrane. The bundles are compact in the body of the cytosol but start to unravel about 3.4 μm from the plasma membrane whereupon individual filaments course through the apical portion of the cell to insert on the plasma membrane. Some exocytotic activity of the plasma membrane is evident. The mantle-cavity epithelium is also simple columnar in construction and is supported by a dense collagenous connective tissue. The basal lamina is thrown into a series of folds which gives this epithelium a convoluted appearance. Two cytotypes are in evidence as well as numerous hemocytes, some enclosing renal concretions. The apical portions of Cytotype 1 exhibit considerable exocytotic activity.

DEVELOPMENT OF AN EXPERIMENTAL, TROPICAL SHELLFISH HATCHERY FOR THE CULTIVATION OF COMMERCIALLY IMPORTANT MARINE BIVALVES IN PANAMA

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Reductions in stocks of commercially important bivalves in Panamanian waters over the last decade have had a serious impact on the national economy and local communities that depend on these molluscs for food and economical support. Scientists at the University of Delaware and the University of Panama are attempting to replenish shellfish populations through a collaborative effort aimed at establishing a tropical hatchery at the University of Panama's Center for Marine Science and Limnology. Species selected for hatchery culture include the clam *Protothaca asperrima* (Sowerby), the Pacific calico scallop *Argopecten circularis* (Sowerby), and the mangrove oyster *Crassostrea rhizophorae* (Guilding). Thus far this interaction, sponsored in part by the Delaware - Panama Partners of the Americas and the University of Delaware Title XII Program, has resulted in exchanges of information on hatchery technology and personnel between the two institutions. We report initial results of studies of spawning and rearing larvae of these molluscs in Panama using food provided from natural waters and cultures of selected tropical algae.

EFFECTS OF MSX DISEASE ON OYSTER HEMOLYMPH PROTEINS

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Total protein concentration and SDS electrophoretic banding patterns were compared in mortality-resistant and mortality-susceptible oysters exposed to the pathogen MSX (*Haplosporidium nelsoni* [Haskin, Stauber and Mackin]). The objective was to determine whether hemolymph characteristics that are associated with MSX infection or with disease resistance could be detected against a background of individual, seasonal, and habitat variability. Hemolymph protein concentrations in uninfected oysters were highest (mean 20-25 $mg \cdot ml^{-1}$ ovalbumin equivalents) in early fall, and lowest (10-14 $mg \cdot ml^{-1}$) in summer. MSX did not alter these levels as long as parasites were confined to the gill; however, serum protein decreased markedly, and in proportion to infection intensity, when MSX became systemic. The most heavily parasitized individuals had concentrations of only 3-4 $mg \cdot ml^{-1}$. This parallels the finding that MSX-associated mortality rarely occurs until infections become systemic. The data suggest that MSX-induced loss of circulating proteins contributes to the deaths of infected oysters. Electrophoretic analysis of hemolymph revealed a tremendous

amount of individual and seasonal variability, and no consistent correlation of protein banding patterns with MSX parasitism, with resistance to MSX-kill, or with total protein levels. There is some evidence that high molecular-weight proteins appear in the hemolymph during periods of shell closure so that prebleeding treatment of oysters may be an important influence on electrophoresis results.

STRUCTURE AND MINERALOGY OF LARVAL AND POSTLARVAL SHELLS OF *MYTILUS EDULIS* LINNE AND *ISCHADIUM RECURVUM* (RAFINESQUE)

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Observations of two species in the family Mytilidae have revealed that the morphological onset of the change from an aragonitic larval shell to a shell with a calcitic outer layer is not necessarily concurrent with settlement. Mature specimens of the blue mussel *Mytilus edulis* and the hooked mussel *Ischadium recurvum* were spawned and the resulting larvae cultured at temperatures and salinities similar to those at the sites of collection of the adult organisms. Larvae and postlarvae were sampled regularly until the mussels were 1 mm long; the shells were cleaned with a 5.25% solution of sodium hypochlorite to remove the soft tissues and periostracum. Both X-ray diffraction and staining with Feigl's solution were used to differentiate between aragonite and calcite in the outer shell layer. Scanning electron microscopic techniques were utilized to document postlarval changes in shell microstructure. In both species, the lengths of the larvae at settlement were within the range of 200 to 300 μm. In *Mytilus edulis* a marked prodissoconch-dissoconch boundary and a shift from an entirely aragonitic larval shell to the dissoconch shell with an outer calcitic layer occurred at the time of settlement. In contrast, *Ischadium recurvum* lacked a distinct prodissoconch-dissoconch boundary at settlement. A transition from aragonite to calcite in the outer shell layer occurred when the mussels were 500 to 700 μm long. This transition was correlated with dramatic changes in shell structure and surface morphology. Because settlement and the onset of metamorphosis are not always coincident with striking changes in shell surface morphology, microstructure, or mineralogy, caution should be exercised in ecological and paleontological interpretations of such early morphological features.

THE USE OF THE TOADFISH *OPSANUS TAU* (LINNAEUS) AS BIOLOGICAL CONTROL OF CRABS PREYING ON JUVENILES OF THE HARD CLAM *MERCENARIA MERCENARIA* (LINNE) IN FIELD CULTURES

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Predation by crabs is a serious problem in the field culture of hard clams. The oyster toadfish, a hardy and abundant fish that feeds primarily on crustaceans, was tested as a biological control against crabs. Hard clams (3-mm shell length), planted in cages under gravel aggregate, were protected against crab predation by toadfish. Crabs found in the experimental area were the blue crab *Callinectes sapidus* Rathbun and the mud crabs *Neopanope texana sayi* (Smith) and *Panopeus herbstii* Milne-Edwards. After 6 weeks, 49.2% of the hard clams in cages with toadfish had survived, while only 1.6% survived in cages without toadfish. Predation by crabs in bottom cultures of hard clams that are protected by a combination of gravel aggregate and plastic nets may be reduced by placing toadfish under the nets.

EARLY LIFE HISTORY OF THE ARCTIC WEDGE CLAM *MESODESMA ARCTATUM* (CONRAD)

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The arctic wedge clam is a subtidal bivalve inhabiting continental shelf waters to depths of 90 m from Chesapeake Bay to Greenland. Sexually mature adults were induced to spawn with an injection of 0.3 ml of 2-mM serotonin (5-hydroxytryptamine, creatinine sulfate complex) into the gonad. Larval and early postlarval stages were cultured under laboratory conditions using standard bivalve rearing techniques. Larval cultures were maintained at 20°C and salinities varied little from 32‰. Fertilized egg diameters ranged from a minimum of 60 μm to a maximum of 68 μm. A trochophore stage, which was attained 24 to 48 h after fertilization, matured into a planktotrophic, straight-hinge veliger stage within 72 h. The smallest shelled larva had length and height dimensions of 75 and 65 μm, respectively. Prodissoconch I lengths ranged from 75 to 90 μm (n = 50). A pediveliger stage was first observed on day 15; minimum shell length at this stage was 125 μm. Settlement was first observed on day 18. The smallest metamorphosing larva had a shell length of 190 μm. Prodissoconch II lengths, as determined from measurements made on 50 early postlarval specimens, ranged from 190 to 265 μm. Postlarval specimens were

held in shallow troughs (no supplemental feeding) with flowing, unfiltered seawater at ambient temperatures (-1° to 20°C) and salinities (28 to 32‰); shell lengths of juveniles ranged from 3.5 to 14 mm approximately 6 mo after metamorphosis.

THE HEART OF THE NORTHERN HARD CLAM: ITS ENDURING ROLE IN NEUROPHARMACOLOGICAL RESEARCH

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For more than half a century, the isolated heart of *Mercenaria mercenaria* (Linné) has been used to investigate the effects and mechanisms of action of neurotransmitters and neuropeptides. About 30 yr ago, the heart was shown to be innervated by cholinergic cardioinhibitory and serotonergic excitatory neurons. Indeed, the "venus heart" provided one of the earliest examples of serotonergic transmission, and it figured prominently in studies of the effects of serotonin and related indole analoges, such as LSD. Since the clam heart is extraordinarily sensitive to acetylcholine (ACh) and serotonin, it serves as a convenient bioassay for these agents. Recently, a cardioexcitatory agent, first found in clam ganglia, was identified as the neuropeptide: phenylalanyl-methionyl-arginyl-phenylalanine amide (FMRFamide). The mode of action of FMRFamide on the hearts of *Mercenaria* and related species, and on other molluscan preparations, has been thoroughly described and has initiated a world-wide investigation into the actions and distribution of this new molluscan peptide family. Excepting FMRFamide and its analogs, most of the known neuropeptides are inactive on the isolated clam heart; however, and unexpectedly, the red-pigment concentrating hormone of prawns, and the chemically related adipokinetic hormone of locusts, are potent excitors of this preparation. The effect is especially intriguing in that only half of the hearts tested responded to these peptides. The mechanism of this unusual action is not known, but it is *not* related to sex or subspecies. Clam ganglia contain a red-pigment concentrating factor active in crustaceans; but its physiological significance in the clam is unknown.

GROWTH OF JUVENILE OYSTERS AND CLAMS ON HETEROTROPHIC MICROFLAGELLATES

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Heterotrophic microflagellates are potential bivalve foods that may be more easily cultured in shellfish hatcheries than conventional algal strains. The growth of juvenile oysters (*Crassostrea virginia* [Gmelin]) and clams (*Mercenaria mercenaria* [Linné]) that were fed five species of heterotrophic microflagellates was examined. Microflagellates, which ranged in length from 2.6 to 7.8 μ included *Paraphysomonas vestita*, a colorless chrysophyte, two bodonids, and a choanoflagellate. Microflagellates were raised on estuarine bacteria cultured on brewer's condensed solubles (BCS), a syrupy byproduct of the brewing industry. Oysters, whose initial weights ranged from 0.5 to 1.0 g, were raised in plexiglass chambers with flowing, filtered estuarine water into which various concentrations of the microflagellates were metered. Oysters grew on diets of *P. vestita*, but no growth occurred with the other microflagellate isolates. Enriched cultures, containing a mixture of microflagellates and bacteria, gave inconsistent results. In another experiment, groups of 10 clams, with initial weights ranging from 5.3 to 6.8 g, were raised in 1-*l* beakers. Clams exhibited growth on diets of *P. vestita* and the unidentified colorless chrysophyte but not with the bodonids or the choanoflagellate. Oysters and clams that were fed comparable quantities of the alga *Tetraselmis suecica* exhibited greater growth than those fed microflagellates. Microflagellates, however, produce significantly greater growth rates than phytoflagellates and can be raised in the dark at high cell densities.

THE STATUS OF THE HARD-CLAM FISHERY IN NEW JERSEY

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The fishery for the hard clam (*Mercenaria mercenaria* [Linné]) in New Jersey operates in coastal bays from Raritan Bay to Cape May. Production has fluctuated between 453,600 and 2,268,000 kg (1 to 5 x 10^6 lb) per year since 1889 when records began. Peak harvests in the late 1940's and early 1950's were followed by a sharp downward trend, coincident with the closing of clam beds in contaminated areas. Since 1960, the reported harvest has been between 453,600 and 1,360,800 kg (1 to 3 x 10^6 lb). Because the price of clams increased from about $1.10 to $5.50 per kilogram ($0.50 to $2.50 per pound) between 1967 and 1983, however, the total value of the harvest has increased. The 1983 harvest of 590,000 kg (1.3 x 10^6 lb) valued at $3.3 million. Since 1970, clammers have relaid stocks from restricted (70-700 MPN·100 ml^{-1}) and condemned (700 + MPN·100 ml^{-1}) areas to privately leased grounds in approved waters. Clams are marketed after 30 days at temperatures above 10°C. Relaid clams account for 5 to 18% of each year's total production. In July 1983, a depuration plant opened

which can process clams from restricted water only. Between July and December 1983, the depuration plant handled approximately 3 million clams (about 10% of the annual production and equal to the number of relaid clams). Two individuals have operated hatcheries to provide seed for their own grow-out grounds for about 10 yr; however, they produce less than 1% of the total New Jersey harvest. A third hatchery/grow-out operation started this spring.

SETTING OF THE AMERICAN OYSTER *CRASSOSTREA VIRGINICA* (GMELIN) IN THE JAMES RIVER, VIRGINIA: THE TEMPORAL-SPATIAL DISTRIBUTION

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Setting of oyster larvae was monitored at 11 stations in the James River, VA, from 1963 to 1980. Strings of oyster shells, suspended 0.5 m off the bottom, were changed weekly during the setting season (June - October) and the numbers of attached oyster spat were counted. Setting patterns were similar in two ways to those described prior to 1960 (before the onset of *Haplosporidium nelsoni* [Haskin, Stauber and Mackin] in Chesapeake Bay): setting intensity (mean number of spat per shell) was greater at stations in the lower than upper estuary, and on the average, 60-80% of the annual set at each station occurred during a 6-wk period from mid-August through September. However, annual setting intensity from 1963-1980 was lower than recorded previously and annual sets occurred as a series of discrete pulses rather than continuously throughout the season. Pulses were 1 to 2 wk in duration and separated by a 1- to 2-wk period of diminished setting intensity. Cross-correlation analysis of annual setting patterns among stations revealed three zones in the James River: the upper estuary and entire southwest side, the lower estuary, and a mid-estuary transition zone. Setting pulses tended to be synchronous at stations within each zone, but occurred 1 to 2 wk later at stations in downriver than in upriver zones. Timing of setting pulses within and between zones were related to known aspects of water circulation in the James River. Pulse setting may be related to the absence of strong vertical salinity gradients accompanying fortnightly stratification-destratification.

ESTIMATING THE GROWTH OF LARVAE OF *CHIONOECETES* SPP. IN THEIR NATURAL PLANKTONIC ENVIRONMENT

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Feeding success during planktotrophic larval development is one of many factors which may influence recruitment to populations of decapod crustaceans. Feeding success is measured against estimated required daily ration, but the latter estimate is based, among other things, on knowledge of growth rates. Reliable estimates of growth rate of predatory meroplankton may be difficult to obtain from laboratory studies, and the results of such studies should be compared with estimates from natural populations whenever possible. A method for doing this is described using molt staging of zoeae of, Tanner crabs, *Chionoecetes bairdi* Rathbun and *C. opilio* (Fabricius), collected from the plankton of the southeastern Bering Sea. A comparison of various equations for estimating average daily growth rates and for describing actual patterns of zoeal growth is made.

FEEDING TRAILS WITH JUVENILES OF THE AMERICAN LOBSTER *HOMARUS AMERICANUS* MILNE-EDWARDS USING VARIOUS LEVELS OF DIETARY ASCORBIC ACID AND COPPER

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Replicate groups of juvenile lobsters were fed semipurified diets supplemented with copper and l-ascorbic acid (AsA). Following a fractorial design, supplemented Cu levels were 0, 0.016, 0.16, and 1.6 $g \cdot kg^{-1}$ dry diet and AsA levels were 0, 1.2, and 12 $g \cdot kg^{-1}$ dry diet. A diet based on crab protein was used as a reference diet. The feeding trials ran for 16 weeks at 20°C. At termination, tissue samples were removed for analyses. All diets containing 1.6 $g \cdot kg^{-1}$ of Cu resulted in growth depression, failure to molt, and high mortalities. Possible interactions between AsA and Cu are discussed.

RIVER FLOW AND SPAT RECRUITMENT ON DELAWARE BAY SEED OYSTER BEDS

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Larval abundance, shell-bag set (set potential), and bottom set (recruitment) have been determined for Delaware Bay seed oyster beds every year since 1954. Annual means for these measures were correlated with each other and with May-July Delaware River flow. The object was to determine whether there was a particular stage in the recruitment process that was most sensitive to river flows. Larval densities, set potential, and spat survival varied greatly from year to year. One long-term trend was evident: survival of spat on the lower seed beds, which had been out of production for 20 or more years, increased several-fold during the late 1960's, concurrent with sustained, elevated river flows. There was generally good correlation between numbers of early- and late-stage larvae and between late-stage larvae and set potential. The relationship between set potential and bottom recruitment was less strong. River flows were positively correlated with larval abundance and to a lesser extent with recruitment. The largest larval broods and the best recruitment, as well as the highest river flows, followed Hurricane Agnes in 1972. The data suggest that early summer Delaware River flow influences oyster recruitment in two ways: 1) long-term changes (over several years) affect larval settlement and spat survival by controlling predators and competitors, and 2) short-term (annual) fluctuations influence larval production (gametogenesis and spawning).

DEVELOPMENT OF ARTIFICIAL DIETS FOR MARINE BIVALVES

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The culture of marine suspension-feeders, including clams, is mainly dependent on algae as a source of nutrients. Algae is an expensive and undependable food for commercial enterprises and its complex and variable composition makes nutritional studies difficult to interpret. One way of overcoming these difficulties is to use artificial diets as a food source; however, problems in both presenting microparticulate foods and in determining their optimum dietary composition, have hindered the development of satisfactory artificial diets for marine suspension-feeders. Recent advances in the development of artificial diets for suspension-feeders are reviewed. The application of microencapsulation technology and the use of dispersants and antibiotics to control food particle clumping and bacterial growth are discussed, together with the results of growth experiments with the American oyster *Crassostrea virginica* (Gmelin) that received microencapsulated diets.

SOME OBSERVATIONS ON THE LONGEVITY OF THE HARD CLAM *MERCENARIA MERCENARIA* (LINNÉ)

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In the late 1940's and early 1950's a series of mark-recapture experiments was conducted by Thurlow C. Nelson and Harold H. Haskin to assess the growth of northern hard clams on Delaware Bay mud flats adjacent to Rutgers' Oyster Research Laboratory (Cape May County, NJ). Two specimens from these experiments were recovered alive on 7 September 1980. The growing shell margin of one of these specimens (#1; shell length at sampling = 87 mm) had been notched with a hack saw between March and December 1947 (shell length at notching = 49 mm). The growing shell margin of the second specimen (#2; shell length at sampling = 99 mm) had been notched with a small triangular file between March 1948 and December 1951 (shell length at notching = 58 mm). Examination of polished shell sections (cut along the axis of maximum growth) of specimens #1 and #2 revealed, respectively, 33 and 30 dark (translucent) bands within the postnotch regions of the outer and middle shell layer of each specimen. The presence of light (opaque) shell material at the growing margin of each of these specimens is consistent with the observations of other workers that indicate formation of dark (translucent) bands in this species during the winter months in New Jersey waters. Interpretation of surface and internal growth patterns of the prenotch shell regions of the two specimens suggests that each was approximately 3 yr old at the time of notching. The resulting age estimates of 36 and 33 years for these specimens are, to the best of our knowledge, the oldest reported to date for this species from long-term monitoring studies.

SEDIMENT PREFERENCES AND MORTALITY IN YOUNG SURF CLAMS (*SPISULA SOLIDISSIMA* DILLWYN)

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A study was conducted in experimental field trays and by SCUBA observations and sampling of setting preferences and mortalities in young surf clams off the coasts of western Long Island, New York, and northern New Jersey. Surf clam pediveligers select coarse sand over fine sand and avoid mud when settling in the bottom. Settlement densities of juvenile clams ranged from about $100 \cdot m^{-2}$ to at least $8000 \cdot m^{-2}$, varying among locations and years, on bottoms within about 3 km of the two coasts. About 10% of the clams died immediately after settling, apparently from physiological causes. Juveniles of the common northern moon snail *Lunatia heros* (Say) settled on the bottoms simultaneously with the juvenile clams and began to consume them. Mortalities caused by the moon snails were 12.5% in a 2-wk experimental period in 1983. Periodic sampling of the clams and SCUBA observations in the area over a period of years showed that all but an extremely minute number of juvenile clams were killed by predators, especially two crab species: *Ovalipes ocellatus* (Herbst) and *Cancer irroratus* Say. In the summer of 1976 most benthic animals including surf clams and crabs were killed by hypoxic water in an area of about 9670 km^2 of the continental shelf off New Jersey. In the fall of 1976 clams set in an area along the southern half of the New Jersey coast in a band that averaged a few kilometers wide lying close to shore; many survived and now comprise a large stock of market-size clams. We believe that the clams had exceptionally high survival because the crabs had been killed.

THE STATUS AND POTENTIAL OF PUBLIC AND PRIVATE CULTURE OF THE HARD CLAM *MERCENARIA MERCENARIA* (LINNÉ) IN NEW YORK

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Juvenile ("seed") hard clams are being produced by 4 commercial hatcheries on Long Island. In recent years, seed from those hatcheries and from hatcheries in Maine and Massachusetts have been planted on public grounds by 7 Long Island towns. Those plantings, ranging in scale from 100,000 to 3,000,000 clams, are being carried out in an effort to supplement natural recruitment. Most town programs include some type of nursery system designed to grow the clams from their 0.5- to 6.0-mm size at purchase to 15 to 20 mm at planting. Although the programs have been carried out for several years, their contribution to the fishery has not been rigorously determined. Our preliminary evaluation of those programs and experimental plantings on Long Island suggest that the town programs are much too small to make a significant quantitative contribution to the public harvest. The plantings might be useful to establish self-sustaining populations at specific sites. Private, hard-clam culture, involving rafts, floating stacks of trays, bottom boxes, etc., has been carried out on Long Island by Blue Points Co., Inc., F.M. Flower Co., and Shellfish Inc., among others. Nursery costs, the lack of suitable underwater land, and opposition from baymen continue to inhibit the expansion of private clam culture on Long Island.

FACTORS AFFECTING MOLTING SUCCESS OF THE BLUE CRAB *CALLINECTES SAPIDUS* RATHBUN HELD IN CLOSED, RECIRCULATING SEAWATER SYSTEMS

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A commercial, closed, recirculating seawater facility that utilized biological filters for control of nitrogenous metabolites is described. Loading rates of 1000 crabs were maintained in each system. Parameters (NH_3-N, NO_2-N, NO_3-N, pH, dissolved O_2, salinity, temperature, alkalinity) that affect molting survival and commercial operating procedures that affect water quality were monitored for a 2-month period and are presented. Alkalinity and pH values declined in the systems, demonstrating a limited buffering capacity of the filter. Values that exceeded 350 mg/L NO_3-N were observed with no apparent effects to the crabs. Increased molting mortality occurred when concentrations of nitrite that approached 1.6 mg/L NO_2-N accumulated in the systems. Nitrite accumulations were associated with depressed oxygen levels that were induced by peak system loadings or equipment failure. Molting rates in ex-

cess of 95% were observed and in general, acceptable water quality was maintained by the filters.

MERCENARIA IN SOUTH CAROLINA: WILDSTOCK FISHERY AND COMMERCIAL MARICULTURE

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The wildstock, hard-clam fishery in South Carolina began at the turn of the century but remained a small localized fishery until fairly recently. The initiation of mechanical harvesting in 1973 greatly increased annual yields. Latest available annual statistics (September 1982-May 1983) indicate that the wildstock industry now accounts for 3 to 5% of the national harvest and for the first time exceeds the value of the state's oyster (*Crassostrea virginica* [Gmelin]) landings. A summary of fishery techniques and historical statistics is presented for the state's wildstock hard-clam fishery. Hard-clam mariculture began in South Carolina with tray growout experiments in the mid-1970's. These led to a commercial-scale project involving both public and private resources. The cooperative project utilized a three-step culture protocol: nursery culture to field planting size, high-density primary field growout, and lower-density secondary field growout to minimum market size. A discussion of the progress enjoyed by the cooperative project, its production to date, as well as a summary of the potential of, and constraints to, hard-clam mariculture in South Carolina is presented.

GAMETOGENESIS IN A POPULATION OF THE HARD CLAM *MERCENARIA MERCENARIA* (LINNÉ) IN NORTH SANTEE BAY, SOUTH CAROLINA

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Adult hard clams, *Mercenaria mercenaria*, were sampled monthly between December 1977 and February 1979 and semi monthly from March to June 1981, from subtidal populations in North Santee Bay, South Carolina. Gonad development was monitored through standard histological methods and resulting slides were examined with light microscopy at 100, 200, 400, and 1000 x magnifications. Observed gametogenic progression was best categorized by five stages or phases of development: inactive, ripe, spawning, partially spent, and spent. Both male and female clams displayed a complex progression of gametogenesis. Gonadal tissue was not uniformly dominated by clearly defined, distinct stages. Instead gonads routinely exhibited several stages simultaneously and progression was documented through slow shifts in domination of stages in gonad tissue. Spawning in the population occurred continuously over a 6-month period (May to October) with at least two apparent peaks of spawning activity in the summer months. The stages of gametogenesis encountered in this study are described for both male and female clams and seasonal progression of gonad development in both sexes is discussed.

CULTURE OF THE HARD CLAM *MERCENARIA MERCENARIA* (LINNÉ) IN COMMERCIAL-SCALE, UPFLOW NURSERY SYSTEM

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Upflow nursery systems for culture of bivalve molluscs and seed are attractive alternatives to traditional raceway systems. The potential benefits include maximization of space utilization, low construction cost, ease of maintenance, and operational longevity. A commercial nursery facility for raising hard-clam seed in South Carolina employs upflow culture instead of traditional raceway systems. This paper reports results from the first year of operation of this upflow nursery system. Seed growth is analyzed in relation to seed density, water flow, and environmental parameters. Growth rates of seed from three different broodstocks is reported. Performance of passive and active upflow systems are compared. Results are compared with those from raceways and from an experimental-scale, passive upflow system.

AN OVERVIEW OF SOME ASPECTS OF HARD CLAM BIOLOGY

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This review covers a number of general aspects dealing with

hard clam biology. Among the subjects covered are the detrimental effects of oil on clams, and the clams' responses to other pollutants. Also discussed are shell uniformity and the ability of clams to remain closed for weeks out of water. Other biological topics reviewed include the production of antitumor agents by clams, the effects of certain neurosecretions on clam hearts, the effects of environmental factors on shell growth increments, and the functioning of the catch-muscle mechanism. General fishery topics covered include techniques for preventing predation, and certain aspects of a new hard-clam fishery in the Santee River delta in South Carolina following the diversion of Santee River water to the Cooper River.

SURVIVAL OF THREE SPECIES OF QUAHOG CLAMS (*MERCENARIA* SPP.) IN REFRIGERATED STORAGE

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Differential shelf-life for various quahog clam species that are harvested in Florida has been demonstrated at three distinct refrigeration temperatures: 4°, 10°, and 15°C. The Northern quahog *Mercenaria mercenaria* (Linné) harvested along the east coast has a significantly longer shelf-life than the Southern quahog *Mercenaria campechiensis* (Gmelin) and the Texas quahog *M. mercenaria texana* Dall harvested along the Gulf coast pan-handle region. The survival response across all storage temperatures was significantly longer for all clam species harvested during January through April as compared to harvest in June through August. All species in 4°C refrigeration experienced a stress condition which would be interpreted as death by commercial standards. Survival was longer in 10° and in 15°C, but potential adverse microbial consequences and objectionable odors resulting from single deaths would preclude use of this storage temperature. Fecal coliform and aerobic plate counts (35°C) of live clams remained relatively constant during storage; however, aerobic plate counts conducted at 25°C showed a marked increase for clams stored at all temperatures. Further considerations with use of initial, temporary wet storage in ambient and refrigerated water for acclimation offered advantages, but do not appreciably extended subsequent refrigerated shelf-life.

APPLICATION OF EXPERIMENTAL HYPOTHESIS-TESTING TO HARD-CLAM MANAGEMENT PROBLEMS IN NORTH CAROLINA

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Field experiments with *Mercenaria mercenaria* (Linné) in North Carolina provided evidence to support various habitat-specific strategies. Seagrass beds provide some natural refuge for hard clams from predatory whelks. If mechanical clam harvesting is prohibited in seagrass beds, these habitats can shelter older, economically less valuable clams to serve as a "spawning pump" for heavily harvested areas. Mechanical clam harvesting in seagrass beds causes long-term damage to the seagrass and does not enhance settlement success of hard clams. Consequently, the benefits of habitat-specific clam management that prohibits mechanical harvesting in seagrass beds outweight the costs, as judged from field experiments in North Carolina.

COMMERCIAL MARICULTURE OF *MERCENARIA MERCENARIA* (LINNÉ) AT AQUACULTURAL RESEARCH CORPORATION, DENNIS, MASSACHUSETTS

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During May and June, 5- to 8-mm, hatchery- produced quahog seed is planted in a field nursery. Two types of nurseries are used: surface suspended and bottom suspended trays. During September and October, nurseries are harvested. Seed-size ranges between 15 and 25 mm, and recovery is between 90 and 95%. Within 48 h of harvest, seed is bottom planted in an intertidal area and covered with 0.5-in. Conwed mesh. Field grow-out including nursery time requires 2.5 to 3 growing seasons, and a recovery of 65% is expected.

PREVALENCE OF OYSTER LARVAL PATHOGENS ON SHELL SURFACES OF ADULTS OF THE AMERICAN OYSTER *CRASSOSTREA VIRGINICA* (GMELIN) FROM TWO SITES IN LONG ISLAND SOUND

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From 1979 to 1981 an inshore study evaluated the bacterial flora of four oyster beds in Long Island Sound and showed that more larval pathogens were associated with a Stratford oyster bed. This initiated a comparison between Stratford, a historically poor setting area, and a highly productive area in New Haven. During a 14-month period these two Connecticut beds were examined to determine the prevalence of pathogenic bacteria on oyster shell surfaces. At approximately bimonthly intervals, oyster shells were collected, aseptically plated on shipboard, and incubated to determine total colony-forming units on marine bacterial media. All isolates were identified to genus and tested in bioassays to determine their pathogenicity to oyster larvae. In larval cultures with isolates deemed pathogenic, mortality ranged from 60 to 96%; results were averaged from three experiments. Of the 219 bacterial isolates collected at New Haven, two (0.9%) were pathogenic. Five of 167 isolates collected at Stratford (3.0%) were pathogens. Although the numbers of these organisms from the two areas did not differ greatly, the present study suggests that there may be some positive association between the presence of larval pathogens on oyster shell and the success of natural oyster set.

SEASONALITY OF FACTORS RESPONSIBLE FOR MORTALITY OF THE NORTHERN BAY SCALLOP *ARGOPECTEN IRRADIANS IRRADIANS* (LAMARCK)

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A population of northern bay scallops in the Poquonock River, Connecticut, was studied in order to eveluate the relative importance of factors responsible for mortality at different times of the year. Estimates of invertebrate predation intensity were derived from recovery of marked scallops released monthly in the estuary. Other factors causing mortality, including burial by shifting sands, suffocation by algal overgrowth, exposure via stranding at low tide, and salinity-temperature effects, were investigated via observation of the natural population and/or monitoring of bay scallop survival in pearl nets. Preliminary results indicate that during the winter months, crustacean and gastropod predation on bay scallops is virtually nonexistent; but predation becomes the dominant cause of mortality as water temperatures rise in the spring and summer. Burial and suffocation appear to represent potentially significant agents of mortality in certain locations during the winter, but do not seem to be as important in warmer months when scallops are more active. Exposure of scallops to the air via stranding at low tides is probably most serious in winter and summer, but may lead to scallop mortality throughout the entire year. Mortalities that result directly from exposure to extreme water temperatures and salinities are sporadic in the Poquonock River, but can cause drastic reductions in population size in rare instances.

STRATEGY FOR PROFIT MAXIMIZATION: FEED AND ENVIRONMENTAL QUALITY

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Aquatic system productivity (growth rate, conversion efficiency, and mortality) is, at least in part, dependent upon feed and environmental quality. Management strategy to maximize short- and/or long-term profits must trade-off the incremental revenue against incremental costs associated with varying feed and environmental quality. Strategies to maximize profit will not always be consistent or compatible with strategies to maximize productivity. A profit maximization model has been developed which considers feed cost, oyster growth rate, oyster conversion efficiency, and oyster mortality, along with selected business characteristics of a company. Growth response of the American oyster *Crassostrea virginica* (Gmelin) fed various combinations of algal and nonalgal feeds, with and without kaolinite particles, was studied. It is shown that maximum profit, in many instances, results with diets where a portion of the algae is replaced with nonalgal feeds and kaolinite.

THE ECTOPARASITIC GASTROPOD *BOONEA* (=*ODOSTOMIA*) *IMPRESSA* (SAY): DISTRIBUTION, REPRODUCTION, AND THE INFLUENCE OF PARASITISM ON OYSTER GROWTH RATES

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We examined the impact of parasitism by *Boonea impressa* on oyster growth and relevant aspects of its population ecology to assess what impact *B. impressa* might have on oyster populations. In the laboratory, small oysters (*Crassostrea virginica* [Gmelin]) that were parasitized by natural densities of *B. impressa* produced 75% less new shell than unparasitized oysters. Shell deposition rates of previously parasitized oysters increased significantly after *B. impressa* was removed. *Boonea impressa* preferentially parasitized small oysters (≤ 2.5 cm) in the field, even though a higher percentage of large oysters was available. The snails maintained an aggregated distribution on the oyster reef. The number of *B. impressa* per oyster clump was positively correlated with the number of living oysters per clump; however, some clumps with few or no living oysters had many snails. Reproduction occurred throughout the year with a peak period in May. Recruitment was greatest in July. The reduction in growth rate of parasitized oysters, the snail's propensity towards parasitizing small oysters, and the snail's tendency to be contagiously distributed suggest that *B. impressa* potentially exerts a significant influence on the population structure and health of oyster populations.

OYSTER CULTIVATION METHODS IN NEW SOUTH WALES, AUSTRALIA

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The Australian oyster *Saccostrea commercialis* (Iredale) has been cultivated on the east coast of Australia for over 50 years in an off-bottom process. Spat are caught on sticks, which are placed at a certain height in the intertidal (oyster) zone, and the following process of growing and maturing takes place at similar levels. The growers have, therefore, no problems with either siltation or predators, such as starfish or boring snails because the oysters are exposed to air twice daily for several hours at low tides. Spat catching, rearing and marketing, oyster depuration, as well as problems with parasites and diseases are briefly discussed.

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ABSTRACTS OF TECHNICAL PAPERS

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A REVIEW OF THE RATIONALE AND POTENTIAL OF TRIPLOID SHELLFISH IN THE PACIFIC NORTHWEST

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Triploid animals typically show a reproductive disadvantage relative to diploids because of the difficulties associated with triploid meioses. Therefore, the reduced reproductive potential of triploids could be a major advantage to organisms with high reproductive energy budgets. *Crassostrea gigas* (Thunberg) is one such animal. Triploidy is induced in the newly fertilized oyster eggs by interfering with the normal course of development. This development consists of a completion of the meiotic cycle beginning at diakinesis. Physical or chemical means may be used to interfere with the second meiotic anaphase resulting in a retention of the second polar body of the egg. Triploidy was induced via cytochalasin B and pressure treatments. Reduced gonadal development has been demonstrated in triploid fish species where characteristically the male develops to a greater extent than the female. In triploid oysters (*C. virginica* [Gmelin]) gonadal development is only slightly retarded in both sexes while in softshell clams (*Mya arenaria*) development is substantially retarded in both sexes. In the Atlantic bay scallop *Argopecten irradians* (Lamarck) gonadal development also lags relative to diploids.

EFFECTS OF GROWTH AND MORTALITY DIFFERENTIALS ON PRODUCTION AMONG SELECTED STOCKS OF THE PACIFIC OYSTER *CRASSOSTREA GIGAS* (THUNBERG)

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A selective breeding program to establish stocks of oysters which would have high survival during summer mortality periods was established in 1976. Oysters produced at the University of Washington Shellfish Laboratory, Manchester, WA, have exhibited variable survival and growth. In 1982, in Mud Bay, WA, 24 groups of oysters from our experimental selection project experienced survival of 60 to 90% while the control group of wild nonselected oysters showed less than 40% survival. Mean wet weights of the oyster meats, however, indicated that most of the experimental oysters were smaller than the controls. Inbred groups were particularly small. A production comparison was made by multiplying the mean wet weight by the percent survival. In all cases, except two of the inbred groups, the production potential of each experimental group was higher than the controls. In addition, four of the experimental groups had mean wet-meat weights comparable to the controls and also exhibited high (80 to 90%) survival. Additional breeding experiments have been conducted with these four groups.

THE WEATHERVANE SCALLOP *PATINOPECTEN CAURINUS* (GOULD): A CANDIDATE FOR AQUACULTURE?

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Scallops are a desired animal for culture on the Pacific coast. There are several native scallops as candidates for culture, including the weathervane. An attempt at rearing weathervane scallop larvae was made at the University of Washington Shellfish Hatchery, Manchester, WA, during May and June 1984. Adults were induced to spawn using seratonin. Larvae were reared to metamophosis in 150-*l* tanks at several different temperatures: 12°C and 17°C in one test and 14°C, 16°C, and 18°C in another test. During the most rapid growth, at 18°C, the larvae reached metamorphosis 18 days from straight hinge. Larval survival from straight hinge to pediveliger at 18°C was 17%. Survival rates at all other temperatures was 10% or less. Larvae were placed in a downweller with a bed of crushed oyster shell for metamorphosis. The metamorphosed scallops grew from 360-μm (shell length) at setting to 800 μm in 12 days. At this point they were removed from the crushed oyster shell to a bare screen and subsequently suffered nearly total mortality. Adult weathervane scallops that were held in flow-through tanks of ambient temperature seawater and in lantern nets suffered very high mortalities from unknown causes. In contrast, the pink scallop *Chlamys rubida* (Hinds) and the rock scallop *Hinnies multirugosus* (Gale) that were held in the same conditions exhibited practically no mortality.

DEVELOPMENT OF A PUGET SOUND FISHERY FOR THE OPAL SQUID *LOLIGO OPALESCENS* BERRY

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In an effort to help develop and properly manage a commercial squid fishery in Puget Sound various types of commercial gear were assessed for catch selectivity and efficiency. Hydroacoustics were used to locate squid schools and sounding data were electronically stored for eventual school quantification. Squid specimens were collected for physiological analysis and to follow growth rates. Of the gear currently used and being tested, preliminary results indicate that the use of highly selective, low-cost gear such as brails and jigging machines should be encouraged. We demonstrated that echo traces of opal squid can be identified although confirmation was possible only on spawning grounds. A Biosonics Model 120 integrator (digitizer) will be used to analyze recorded sounding data for school quantification. Dorsal mantle lengths of spawning squid and spawning times suggest that more than one stock of *L. opalescens* resides or occurs in Puget Sound. Starting in October 1984, specimens will be collected to confirm this using electrophoresis and aging of statoliths. Indications are that a minor fishery based on small fishing boats and a few processors is likely to develop.

EARLY DETECTION OF TRIPLOIDS IN EGGS AND LARVAE OF THE PACIFIC OYSTER *CRASSOSTREA GIGAS* (THUNBURG)

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Analysis of ploidy in Pacific oysters was performed during first cleavage by cytogenetic techniques. By fixing eggs during early cleavages mitotic chromosomes can be visualized and counted, thus serving as early indicators of ploidy. Larval oysters (250 - 330 μm) were individually crushed and agitated to disperse cells, then stained with DNA-RNA-specific fluorescent dye. Preparations were presented to the ICP-22 cytofluorograph in liquid suspension. Stained cells were excited by mercury arc lamp and passed through a sensing zone where the intensity of fluorescence was recorded. Presence of triploids was definitively demonstrated despite the low quantity of cells analyzed.

LARGE-SCALE HATCHERY AND NURSERY REARING OF THE PACIFIC GEODUCK CLAM *PANOPEA GENEROSA* (GOULD)

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A pilot hatchery and nursery has been built at the Point Whitney Shellfish Lab to rear Pacific geoduck clams (*Panope generosa*). Techniques developed in the pilot stage will be applied to produce 30 x 10^6 juvenile clams (10-15 mm in length) per year. The juvenile clams will be planted in areas where marketable clams have been harvested by commercial divers. Hatchery and nursery techniques were as follows. Adult clams were conditioned for spawning by being held in running seawater at 9-12°C and fed continuously with *Thalassiosira pseudonana* (3H). Conditioned adults were spawned by exposure to a dense suspension of 3H in seawater at 16°C. Larvae are fed *Chaetocerus calcitrans* (CC) until 200 μm in length and thereafter fed a mixture of CC and 3H. Larvae that were reared at 17° ± 1°C and a salinity of 29 $^0/_{00}$ were competent to metamorphose 18 days following fertilization at lengths of 330-350 μm. Competent larvae set in downwells and postlarvae were reared in upwells to 15 mm in length. The postlarvae were continuously fed 3H.

In experiments, competent larvae were induced to undergo metamorphosis within 12 hours by exposure to tubes of the polychaetes *Spiochaetopterus costarum*, *Diopatra ornata*, *Phyllochaetopterus prolifica*, the supernatant and precipitate from a seawater homogenate of tubes of *S. costarum*, and a 10^{-5} *M* solution of the amino acid L-Dopa. Analysis of variance showed no significant differences in the response of larvae to the above treatments. The above metamorphic inducers had a significantly greater effect than did tubes from the polychaete *Onuphis elegans*, plastic tubes, and controls. These results indicate that an extractable compound of polychaete will induce metamorphosis of geoduck clam larvae. Further work will test the effect of sediments from adult geoduck clam beds and extracts of adult tissues in inducing metamorphosis of competent larvae.

CULTURE OF THE GREEN-LIPPED MUSSEL *PERNA CANALICULUS* (GMELIN) IN NEW ZEALAND: A PERSPECTIVE

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Culture of green-lipped mussels in the Marlborough Sounds of New Zealand enjoys an advanced state of development with the

potential for supplying large volumes of high quality mussels of Pacific-rim countries. Culture methods focus on long-line technology imported from Japan in the early 1970's. A standard, 10 long-line mussel farm occupies a 3-ha lease and may regularly produce 200 m.t. of mussels every 18-mo growing cycle. With about 200 marine farms presently in operation in the Marlborough Sounds, a potential annual production of mussels from this area alone exceeds 40,000 m.t. annually. Biological aspects of this marine mussel include a very rapid rate of growth under suspended culture conditions. Mussels 50 mm in length are produced in less than 8 mo while an 80-to 100-mm mussel is grown in 18 to 20 mo. In addition, *P. canaliculus* remains in reproductive condition for much of the year, maintaining a supply for export of high quality mussels year-round. Problems with New Zealand mussel culture are presently focused on developing methods to reliably induce and maintain natural settlement of mussels onto growing lines. Marketing problems have contributed to a chronic over-supply of mussels for export to markets which remain underdeveloped.

THE RELATIONSHIP BETWEEN GILL AREA AND BODY SIZE, AND A METHOD FOR REMOVAL OF GERMINAL TISSUE IN THE PACIFIC OYSTER *CRASSOSTREA GIGAS* (THUNBERG)

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A morphometric analysis was made on genetically distinct lines of Pacific oysters produced by the School of Fisheries selective breeding program. Total gill area may be a good estimator of body size due to the gills' functions as (a) the filtering mechanism for removing seston from the water column and (b) the site for gas and ion exchange. The use of total gill area as an estimator of body size is discussed in reference to three other body size estimators: dry weight, area of the left valve, and internal volume. Ten oysters from each of 10 experimental families were analysed for these parameters. Statistical analyses of correlations between gill area and the area of the left valve, dry weight, and internal volume, respectively, show that total gill area is highly correlated with internal volume (correlation coefficient = 0.94). A method for separation of germinal tissue from oysters in order to measure gonadal output in oysters is discussed. Fixation by microwave radiation (25 sec for a 50-g oyster) provides a means for preparing an animal for removal of the germinal tissue from a ripe oyster. Variation among different lines of Pacific oysters is observed with 59 - 71% of total dry weight consisting of germinal tissue.

TRIPLOIDY INDUCED IN THE PACIFIC OYSTER BY MEANS OF CYTOCHALASIN B

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Triploidy was induced in *Crassostrea gigas* (Thunberg) by preventing the release of a polar body with the chemical cytochalasin B at three different temperatures. Three treatments, 1 $mg \cdot l^{-1}$ cytochalasin B from 0-15 minutes (I), 15-30 minutes (II), and 30-45 minutes (III) after fertilization, were applied to oysters incubated for the first hour at 18, 20, and 25°C. Percentages of larval survival, growth, and spat triploid-induction were compared among the three temperature experiments and to other molluscan studies to determine the optimal induction conditions for *C. gigas*. Cytochalasin B reduced the number of larvae surviving to the straight-hinge stage in all the treated groups. Larval survival in treatment I for the lower two temperatures was less than 20% of the next best treatment. Larval growth was not significantly different among treated and control groups. Few triploids (0-12%) were induced in the 18 or 20°C experiments for treatments I, while 35% were induced in the 25°C treatment I. The highest induction percentages, 71 and 72%, were produced by treatment III in the lower two temperatures. These experiments demonstrated that cytochalasin B is an effective means for inducing triploidy in *C. gigas*.

PACIFIC RAZOR CLAM MORTALITIES: PATHOLOGY, DISTRIBUTION OF DISEASE, AND APPARENT CAUSE

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During 1983 certain populations of the Pacific razor clam *Siliqua patula* Dixon experienced a decline of over 90% of animals retrievable by well-established population assessment methods. Mortalities were correlated with the occurrence of a severe gill disease of the razor clams. The gill disease was associated with an infection by a unique form of intracellular pathogen referred to as nuclear inclusion X (NIX) and has not been previously reported in the animal kingdom. Infections in clams resulted in reduced gill respiratory surface. The pathogenic organisms occurred within the cell nuclei of non ciliated branchial epithelium. Infected nuclei and cells were extensively swollen; swelling eventually resulted in rupture of the cell membranes. Rupture of the gill cells resulted in secondary

bacterial and fungal infections. The histological evidence strongly suggests that the health of heavily infected animals was compromised by reduced respiratory capacity and secondary infections. Thus, a strong presumptive case exists to link the NIX-associated gill disease and the razor clam mortalities. Disease intensity, rated by examination of histological preparation of over 500 clams, was most severe in clams from the Moclips and Copalis beaches in Washington from July to October 1983. Infection intensity declined from December 1983 through April 1984. Seventy-five percent or greater of juvenile clams recruited into the population during fall 1983 were infected with NIX (from February to April 1984) at a low intensity at which significant lesions were not observed. NIX organisms were found in low numbers in razor clams from northern Oregon to northern Vancouver Island. The organism was not found in clams from the Queen Charlotte Islands or Alaska.

PATHOLOGY AND DIAGNOSIS OF OYSTER VELAR VIRUS DISEASE (OVVD)

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Oyster velar virus disease (OVVD) has been previously described in hatchery-reared larvae of the Pacific oysters *Crassostrea gigas* (Thunberg) and the lesions of the disease have been observed in wild larvae. Resultant hatchery mortalities can be substantial, occur primarily in 170- to 190-μm shell-height larvae and are most severe from April to June. Lesions were studied in over 800 larvae by differential interference contrast microscopy, histological, and electron microscopial methods from four episodes of hatchery disease over a 2-y period. Microscopic examination of live infected animals can be used to presumtively identify individual infected velar epithelial cells. Viral inclusion bodies in the velum were observed by histological methods in 38% of larvae collected from tank bottoms of affected groups and 28% of larvae collected from the water column. Lesions of epithelial cell separation and detachment were also more frequent in tank-bottom samples. Viral inclusion bodies also occurred in oral and esophageal epithelium. Lesions which rendered the velum dysfunctional resulted from the intracellular production of viroplasm prior to viral particle formation. Thus, animals exhibited signs of the disease before infectious viral particles were formed. Strong presumptive diagnosis of the condition can be made on the basis of microscopic and histological examination. These and previous findings suggest that the disease may be widely distributed and vertically transmitted.

ENVIRONMENTAL AND PHYSIOLOGICAL ASPECTS OF GROWTH AND MORTALITY OF *MYTILUS EDULIS* LINNÉ AT TWO LOCATIONS IN BRITISH COLUMBIA

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Populations of cultured mussels at a number of sites in British Columbia and Washington State experience high mortalities of an unknown origin during the second summer of growth. The present study examined the growth and mortality of two test populations of blue mussels on the east and west coasts of Vancouver Island. High mortalities of adult mussels occurred in both test populations over two successive summers (1982 and 1983). Environmental and biological data indicate that the cause of this mortality is complex. The following factors may contribute:

1) Overcrowding;
2) Handling of mussels during the summer;
3) Reproductive stress coupled with the concommittant rebuilding of glycogen stores;
4) Warm water temperatures and/or low salinities during the summer; and
5) A proliferative blood-cell disorder.

It is likely that several of these factors act synergistically and that the relative contribution of each factor varies between grow-out sites. It is recommended that culture methods be adapted such that crowding and handling stresses are minimized. Reproductive and environmental stress can be reduced by appropriate site selection. Sites with cool summer water temperatures, stable salinities, and moderate reproductive output should be given priority. The relationship between mortality and the blood-cell disorder should be examined in further detail.

INVESTIGATIONS OF SPAT COLLECTION ON ARTIFICIAL SUBSTRATES FOR THE SCALLOPS *PATINOPECTEN CAURINUS* (GOULD) AND *HINNITES MULTIRUGOSUS* (GALE) IN WASHINGTON STATE.

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Collection of spat of the weathervane scallop *Patinopecten caurinus* and the rock scallop *Hinnites multirugosus* was attempted

at four locations in Puget Sound and at three locations in the Straits of Georgia from 1 May to 31 October 1983. Project goals were to determine a suitable area or areas to collect scallop spat, the water depth or depth range to achieve maximum setting of spat, the preferred substrate to use for spat collection, and the appropriate time of year to place collectors in the water. Collectors consisting of onion bags that contained one of four substrates (monofilament gillnet [MONO], western red cedar, *Thuja plicata* [CEDAR], black polyethylene film [POLY], and polyethylene Netlon® mesh [NETLON]) were suspended in the water at 4-ft intervals from surface buoys, the Hood Canal Bridge, and piers. All locations had collectors to 40 ft, except one in the Straits of Georgia (Whitehorn; 115 ft) and one in Puget Sound (Hood Canal Bridge; 115 ft). Collectors were replaced at approximately 3-wk intervals (±1 wk) until the end of the study period. Adults of *P. caurinus* were collected at Cherry Point in the Straits of Georgia, and adults of *H. multirugosus* were collected at Bangor Naval Base in Hood Canal at 3-wk intervals (±1 wk) for histological examination of their gonads to determine spawning times. Results of grow-out and electrophoretic studies indicate that approximately 10-15% of spat kept for grow-out from Intalco and Bangor were *H. multirugosus* with the remainder being a species of *Chlamys*. No spat of *P. caurinus* were identified from these samples.

Substantial sets of unidentified scallop spat (greater than 500 spat per collector) were received at Bangor, Bywater Bay, and the Hood Canal Bridge in Puget Sound. Maximum set for all areas was found at Bangor (2600 spat in one collector). The depth range where substantial settlement occurred and depth receiving maximum settlement for these areas were: Bangor - 19 to 41 ft, 29 ft; Bywater Bay - 25 to 35 ft, 25 ft; and Hood Canal Bridge - 51 to 101 ft, 61 ft. No apparent difference in counts were found between MONO and POLY substrates; however, there was an apparent preference for NETLON at Bywater Bay. Biological fouling prior to spat settlement on substrates placed in the water for periods longer than 3 wk may have been responsible for a sevenfold increase in sets received on 3-wk collectors at the Hood Canal Bridge. Maximum settlement occurred at Bangor and Bywater on collectors that were placed in the water from mid-August to the end of September. Peak settlement at the Hood Canal Bridge occurred on collectors placed in the water from mid-July to mid-September. Peak spawning for *P. caurinus* took place between 19 May and 14 June 1983 and for *H. multirugosus* between 17 May and 10 June 1983.

NEW DEVELOPMENTS IN ASSAYS FOR PSP

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Research on Paralytic Shellfish Poisoning (PSP) has revealed a far more complex situation than originally thought, but has also provided a sound basis for developing improved assay methods. No system yet conceived avoids the costs that are associated with collecting and preparing shellfish samples for assay, but several assay techniques currently being developed promise to be more sensitive and accurate than the mouse bioassay.

BEACH SETTING OF EYED OYSTER LARVAE *CRASSOSTREA GIGAS* (THUNBERG) IN PUGET SOUND, WASHINGTON

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Eyed larvae of *Crassostrea gigas* were spread at three intertidal beach locations during low tide exposure to determine if setting would occur. No setting occurred at Bywater Bay when the incoming water temperature was 10°C. Setting occurred at both Lilliwaup and Penrose Point where incoming water temperatures were about 21°C. Laboratory experiments tested setting at 5°C increments from 5° to 25°C. Significant levels of setting occurred at 15°C, 15%; 20°C, 39%; and at 25°C, 67% after 24 h. Further experiments indicate that 20% setting will occur after 6 h at 18°C when larvae are refrigerated prior to setting trials.

POSTHARVEST ESTIMATION OF THE HARVESTABLE BIOMASS OF THE PACIFIC GEODUCK CLAM *PANOPE GENEROSA* GOULD IN AN AREA IN PUGET SOUND, WASHINGTON

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Methods for estimating the harvestable biomass of geoduck clams in a delineated area any time after an initial harvest are presented. Efficient management of the geoduck fishery requires the knowledge of the length of the period between successive harvests of an area. I used variable recruitment rates and variable periods for recovery in my equation for estimation of harvestable biomass. Data for my calculations were from a geoduck population near Dougall Point, Puget Sound, Washington. Geoduck biomass never reached

harvestable levels at a recruitment rate of 0.01 recruits·m^{-2}·yr^{-1}, the recruitment rate estimated for the population over the last 20 yr. A recruitment rate of 0.23 recruits ·m^{-2}·yr^{-1}, the highest rate estimated for the population during the last 100 yr resulted in a harvestable biomass exceeding the preharvest biomass, 2963 g·m^{-2}, in less than 20 yr. Recruitment rates estimated for past data indicate the variability that could occur in the future recovery of a bed to harvestable biomass. Intense sampling for recruits in harvested beds is being completed by the Washington Department of Fisheries. These estimates of recruitment along with improved mortality estimates are needed before the period required to reach a harvestable biomass can be reliably determined.

INTERTIDAL, LONGLINE CULTURE OF *MYTILUS EDULIS* LINNE IN PUGET SOUND, WASHINGTON

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Intertidal, longline systems, similar to those used in Grays Harbor for growing oysters, are presently being used to grow mussels at Race Lagoon, Whidbey Island, Washington. Seed mussels are caught on lines placed on racks in the intertidal zone. Once seeded the 30.5-m (100-ft) lines are attached to rows of PVC pipe inserted in the beach. Growth of mussels with this system is somewhat slower than mussels grown subtidally on rafts in nearby Penn Cove; however, growth to harvest size is 1 year or less. Control of the spring settlement of barnacles seems to be the major obstacle at present.

METAMORPHOSIS OF LARVAE OF THE CALIFORNIA MUSSEL *MYTILUS CALIFORNIANUS* CONRAD

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The present status of our investigations into the problems of rearing large numbers of larvae of *M. californianus* through metamorphosis are reported. The results of an experiment utilizing our knowledge of the ecology of this species is discussed. In this experiment temperature, specific substrates, and vigorous aeration were tested in various combinations in order to discern their importance in the metamorphosis of larvae of *M. californianus*. Success of metamorphosis was best achieved at the lowest temperature tested, 10°C, and with a very vigorous aeration. Presence of a suite of substrates produced no effect in promoting successful metamorphosis.

WHY THE STUDY OF REPRODUCTIVE ENERGETICS IS IMPORTANT TO SHELLFISH GROWERS

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Investigations of the physiological states of various species of bivalves of commercial importance are beginning at the School of Fisheries at the University of Washington. The bioenergetic techniques being employed will provide information to better understand physiological aspects of these species, fitness and vigor in the various habitats of the Northwest. These investigations will be important in providing answers and methods for dealing with such practical problems as mass mortalities as well as allow us to quantify the extent to which these organisms are adapted to their environments and how perturbations of these environments affect growth rate and fecundity. These methods can also be used to assess the physiological states of organisms in the hatchery.

RECENT PROGRESS IN THE ARTIFICAL BREEDING OF FOUR SPECIES OF SCALLOPS

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In the past two years attempts have been made to spawn four species of scallops in the laboratory including two native species, the weathervane scallop *Patinopecten caurinus* (Gould) and the rock scallop *Hinnites multirugosus* (Gale) and two exoties, the Japanese scallop *Patinopecten yessoensis* (Jay) and the Atlantic deepsea scallop *Placopecten magellanicus* (Gmelin). Several methods have been used to stimulate spawning but most consistent results were obtained by injections of 0.4 ml of 2 x 10^{-4} molar solution of serotonin into the adductor muscle. Larvae have been raised in a range of temperatures from 12 to 18°C depending on species but generally at 15°C. Salinities were 28 ± 1%. A variety of algal foods have been used but best growth and survival was obtained using *Chaetoceros calcitrans*, Tahitian *Isochrysis*, and *Thalassiosira pseudonana* (3H). Larvae were raised at initial densities of 1 to

$10 \cdot ml^{-1}$ which decreased to 0.2 to $0.5 \cdot ml^{-1}$ at settlement. Time from fertilization to settlement (pediveliger) has ranged from 20 to 35 days depending on species and environmental conditions. Most success has been achieved with Japanese and rock scallops. Several substrates, including shell, sand, polypropylene rope, and Astro Turf® have been used as cultch but none has proven to be significantly better than others. Larvae have been set under static conditions and in downwelling and upwelling systems, but heavy mortalities have been experienced after settlement.

SEROTONIN INDUCES SPAWNING IN MANY WEST COAST BIVALVE SPECIES

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Intergonadal injection of 0.4 ml of a 2-mM solution of serotonin (5-hydroxytry ptamine creatine sulfate complex, Sigma Chemical) induced spawning in many West coast bivalve species during the breeding season. intragonadal injection of serotonin induced spawning in the males and somtimes females of the following species:

1. *Patinopecten caurinus* (Gould), the weathervane scallop;
2. *Chlamys rubida* (Hinds), the pink scallop;
3. *Panope generosa* (Gould), the geoduck clam;
4. *Hinnites multirugosus* (Gould), the rock scallop;
5. *Saxidomus giganteus* (Deshayes), the butter clam;
6. *Tapes philippinarum* (Adams & Reeve), the Manila clam;
7. *Protothaca staminea* (Conrad), the Pacific littleneck clam;
8. *Crassostrea gigas* (Thunberg), the Pacific oyster;
9. *Siliqua patula* (Dixon), the Pacific razor clam; and
10. *Tresus capax* (Gould), the horse clam.

Serotonin solution was injected into each individual mollusc. Reaction to the injection was swift with spawning most often occurring within 120 min in statistically significant numbers. This represents a new method to artificialy induce bivalves to spawn and it will have important implications in shellfish aquaculture and reproduction research.

INTERTIDAL RACK-CULTURE OF OYSTERS: MAKING THE MOST OF A MUDFLAT LEASE

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Guidelines are suggested for assessing whether an intertidal mudflat is suitable for rock culture and the factors to be considered in selecting the optimum rack height are discussed. Methods for holding single oysters are outlined and compared based on experience in New Zealand and Australia.

HINNITES MULTIRUGOSUS (GALE) AND *CHLAMYS RUBIDA* (HINDS): WILD SEED ACQUISTION IN BRITISH COLUMBIA, CANADA

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Clean, weathered oyster shells in tublar plastic net bags and in lantern nets were placed at various depths to 33 m over a period of 3 yr in Refuge Cove, Waddington Channel, Pendrell Sound, and Trevenan Bay. In 28 individual seed acquisition units, 10926 seeds were taken. In detailed evaluations at Refuge Cove and Waddington Channel the most effective depth for the acquistion of mixed populations of *Hinnites* and *Chlamys* was 16 m from the surface of the water. At this optimun depth the oyster shells in tubular plastic net bags and in lantern nets served equally well as substrate yielding 8.5 seed scallops per oyster shell. Based on periodic pooled counts of seed scallop at all depths, over time, there appeared to be two major spawnings, one observed by counts in March and one observed by counts in September. Observed seed was in the 4- to 18-mm size range (perpendicular to the hinge).

MAXIMIZING SHELLFISH PRODUCTION WITH HIGH TECHNOLOGY

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Potential advantages of highly intensive shellfish production systems cover a wide range of factors from increased labor efficiency to minimizing or eliminating the need for foreshore leases. In British Columbia, an additional rationale for intensive systems, despite their higher energy demands, is a very low ($5.6¢ \cdot KWH^{-1}$)

electrical energy cost. High technology systems have been attempted at several sites around the world and have failed because of scale-up problems not anticipated and lack of back-up systems in the case of power failure. In addition, these attempts have tried to incorporate existing subsystems (i.e., trays primarily) which were originally designed for use in the natural environment and impose serious engineering and economic constraints on a highly intensive system. Several alternative pilot systems for highly intensive shellfish culture have been designed. Some of these designs utilize existing trays and one design is from the "ground up" (i.e., all components are of original design to meet functional specifications). Estimates have been made of capital and operating costs for these pilot plants. Electrical energy operating costs are estimated between 4¢ and 6¢ per oyster depending on details of system design. It appears justifiable to operate these small-scale intensive pilot systems to further evaluate the trade-offs and the uncertainties in the estimates and to determine feasibility for species other than oysters.

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SEASONAL CHANGES IN THE DEPTH-DISTRIBUTION OF BIVALVE LARVAE ON THE SOUTHERN NEW ENGLAND SHELF

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ABSTRACT A limited survey was made of the seasonal change in occurrence, depth distribution, size distribution, and species composition of bivalve larvae at a single station on the southern New England shelf during the period April-December 1981. The data were related to temperature structure of the water column and chlorophyll *a* distribution. Bivalve larvae were most abundant during late August and September at depths greater than 10 m, in water temperatures of 14 to 18°C, and chlorophyll *a* concentrations of $<2 \mu g \cdot l^{-1}$, and at the surface in October in a temperature of 15.5°C and chlorophyll *a* concentrations of $\sim 3.0 \mu g \cdot l^{-1}$. Larvae >200 μm length consisted predominantly of the species *Modiolus modiolus* (Linné), *Arctica islandica* (Linné) and *Spisula solidissima* (Dillwyn). *Modiolus modiolus* was present in the depth range 10-40 m from late July through December with highest concentrations in August through October. *Arctica islandica* was present at 1 to 30 m depth in May and from 20 to 40 m from late July through November. Larvae of *A. islandica* that were captured in May possibly originated from spawning in late 1980; those that were captured in November were first shelled veligers of 110 μm length. Those larvae may form the basis of an overwintering larval population. Larvae of *S. solidissima* were present from late July through October and extended into shallower, warmer waters than larvae of either *M. modiolus* or *A. islandica*.

KEY WORDS: Bivalve larvae, New England Shelf, *Arctica islandica*, *Modiolus modiolus*, *Spisula solidissima*

INTRODUCTION

Franz and Merrill (1980) described the bivalve molluscs of the Middle Atlantic Bight as a mixture of southern and northern species. The southern or Transhatteran species reach their northern limit at Cape Cod and are generally limited to the shallower depths inshore of the seasonal thermocline. The northern species, a mixture of arctic-boreal and boreal species, generally exhibit submergence south of Cape Cod, (i.e., their distribution follows colder isotherms to increasing depth with more southerly latitudes). Recently, one member of this Boreal fauna, the ocean quahog *Arctica islandica* (Linné), was the subject of considerable attention. Mann (1982) and Jones (1981) described the seasonal cycle of gonadal production on the southern New England shelf and offshore New Jersey, respectively. Lutz et al. (1982) expanded the previous work of Landers (1976) to give a comprehensive description of larval and early postlarval development. Mann and Wolf (1983) described the swimming behaviour of the larvae in response to temperature and pressure stimuli. Based on a consideration of an earlier description of seasonal temperature structure of the waters of the Middle Atlantic region by Bigelow (1933) and the data of Landers (1976), Mann (1982) hypothesized, despite the presence of morphologically ripe specimens from March through October, that "larval survival is probably greatest during the months of October and November, which is the time of breakdown of intense seasonal thermocline and precedes the onset of low winter seawater temperatures." The data of Lutz et al. (1982) and Mann and Wolf (1983) support this hypothesis.

Early in 1981 the opportunity arose to make a limited survey of seasonal occurrence, species composition, and depth distribution of bivalve mollusc larvae at a station on the southern New England shelf during the period April-December 1981. While this survey was of insufficient scale to examine both spatial and inter-annual variability, it nonetheless offered an opportunity to examine the hypothesis of Mann (1982) and to provide information on bivalve larvae occurrence on the continental shelf. This report describes the results of the survey.

MATERIALS AND METHODS

During April-December 1981, 14 one-day cruises were made to a station in 43 m of water situated west-southwest of Cuttyhunk Island, MA; west of Gay Head, Martha's Vineyard, MA; and east of Block Island, RI (lat. 72°02'W, long. 41°14'N). The water column at this station exhibits an intense seasonal stratification in water temperature that is representative of southern New England shelf and Middle Atlantic Bight waters (Mann, unpublished data). Adults of *Arctica islandica* are abundant in this area (Merrill and Ropes 1969; Ropes 1978; Fogarty 1981). Depth-specific plankton tows were made, always during the hours of 1030-1430, at 1, 10, 20, 30, and 40 m with a Clarke-Bumpus net (30-cm diameter, 5:1 aspect ratio, 53 μm mesh, 10-min tow duration, 3.7 $km \cdot h^{-1}$ speed). Tows were not replicated. The volume of water that passed through the net was recorded by a vane rotor in the mouth of the net. Volume varied between 9.64 and 10.28 m^3 with a mean value of 9.96 m^3. Collected material was stained with Rose Bengal and fixed with 10% v/v buffered formalin in sea water. Bivalve larvae were subsequently separated under a low power dissecting microscope. During periods of peak abundance plankton samples were split using the apparatus of Drinnan and Stallworthy (1979). Individual larvae were measured in length (anterior-posterior axis) and height (dorso-ventral axis) at 100-X or 400-X

[1]Contribution No 1343 from the Virginia Institute of Marine Science.

on a Leitz compound microscope fitted with an ocular micrometer. All larvae were grouped into three size classes: straight hinge or early umbo larvae of length $<150\ \mu m$; umbo larvae in the length range 150-200 μm; and umbo or pediveliger larvae of length $>200\ \mu m$. Individuals of $>200\ \mu m$ length were identified, where possible, to genus or species using the keys of Chanley and Andrews (1971), de Schweinitz and Lutz (1976), Lutz et al. (1982), and unpublished material kindly supplied by Prof. R. D. Turner (Museum of Comparative Zoology, Harvard University, Cambridge, MA, USA).

On each sampling date water temperature and conductivity were recorded at 5-m depth intervals using a Hydrolab S8000 instrument (Hydrolab Instruments, Austin, TX). During June-December, water samples were collected from the same depths as the plankton tows using a Niskin bottle, a subsample (250-1000-ml depending on concentration) was filtered through a pre-ashed Gelman A/E glass fibre filter and the retained material assayed for chlorophyll *a* by the trichromatic method of Strickland and Parsons (1968). Vertical profiles for temperature and chlorophyll *a* for each date were used to construct, by linear interpolation, time-depth contour diagrams of each.

RESULTS

Hydrographic Observations

Figure 1 illustrates the temperature structure of the water column during the period April-December 1981. Thermal stratification began development in May and intensified through June and July. By the end of July a maximum surface temperature of 20.3°C contrasted with a bottom temperature of 12.3°C and an intense thermal gradient ($0.5°C \cdot m^{-1}$) was evident between 15 and 23 m. During August-September the depth of intense stratification increased as surface temperature gradually decreased. A maximum bottom temperature of 15.0°C was recorded in mid-September. A well-mixed water column was again evident by late October.

Salinities in the range 32.20 to 32.61 $^0/_{00}$ were recorded during the periods of vertical mixing of the water column; variation through the depth of the water column did not exceed 0.15 $^0/_{00}$ on any one collection date. As thermal stratification intensified, surface salinity values decreased to 31.40 $^0/_{00}$ by late August - early September. This lower salinity water extended to the approximate depth of the 17°C isotherm at 25 to 30 m (Figure 1) where it covered water of higher salinity (31.80 to 32.13 $^0/_{00}$). Bottom water typically maintained a slightly higher salinity (by 0.30 to 0.70 $^0/_{00}$) than surface water during the summer thermal stratification.

Chlorophyll Concentration

Figure 2 illustrates chlorophyll *a* concentration in the water column for June-December 1981. A weak chlorophyll *a* maximum in mid-June was observed at 20 m, immediately below the region of intense thermal stratification. Chlorophyll *a* was at a maximum ($\sim 2\ \mu g \cdot l^{-1}$) at 25 to 35 m depth by early August. This was below the region of most intense thermal stratification and corresponded to a thermal range of 13 to 15°C that decreased with depth. In contrast, the water column from 0 to 15 m had both the highest temperature (>19°C) and lowest chlorophyll *a* content ($<0.5\ \mu g \cdot l^{-1}$). Chlorophyll *a* was evenly distributed throughout the water column ($\sim 0.4\ \mu g \cdot l^{-1}$) in late September in spite of some minor thermal stratification from 30 to 43 m. A high concentration of chlorophyll *a* ($>3.0\ \mu g \cdot l^{-1}$) extended to below 20 m ($\sim 2.0\ \mu g \cdot l^{-1}$) in early October following the breakdown of thermal stratification and mixing of the water column. After late October, little change was observed in chlorophyll *a* concentrations either temporally or through depth.

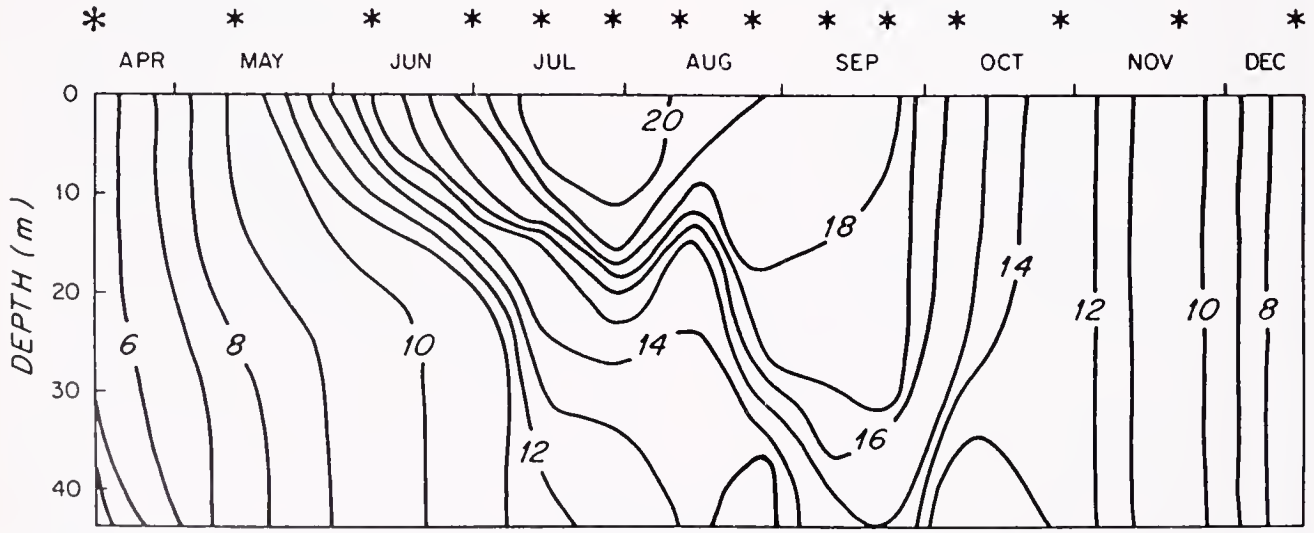

Figure 1. Depth-line isotherm diagram of temperature structure (°C) during the period April-December 1981 based on vertical profiles with a sampling interval of 5 m. (*marks a sampling date.)

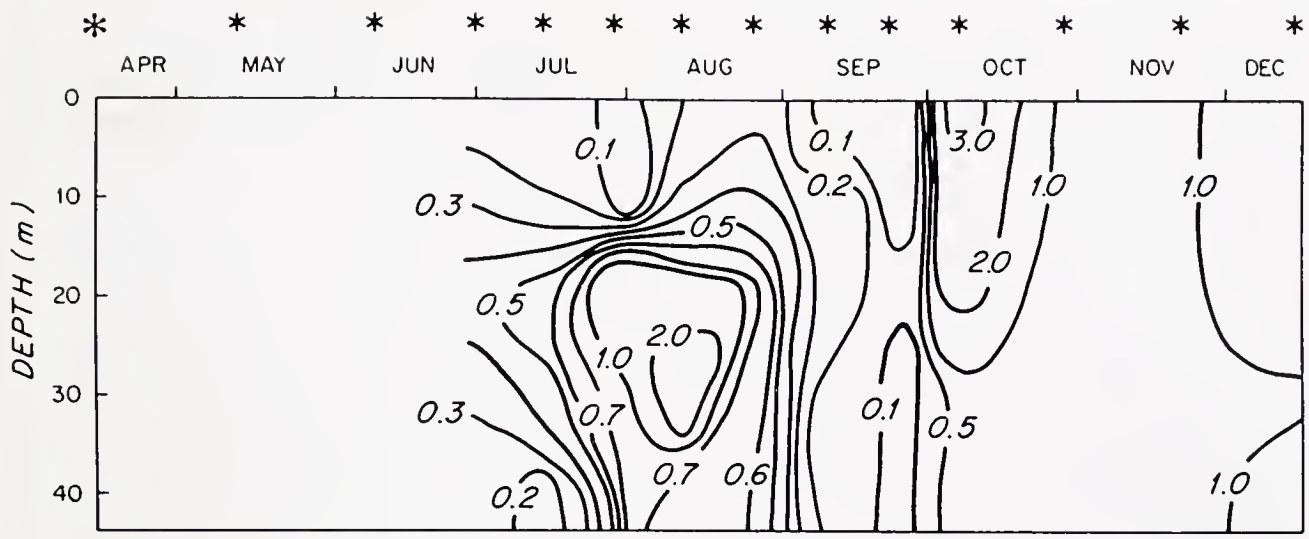

Figure 2. Depth-time contour diagram of chlorophyll *a* concentration ($\mu g \cdot l^{-1}$) for the period June-December 1981 based on Niskin casts at 10 m depth intervals in the water column. (*marks a sampling date.)

TABLE 1.

Seasonal changes in numbers, depth distributionm, and size-class distribution of bivalve larvae on the southern New England shelf during April-December 1981. (ns: no sample collected due to net failure.)

Depth (m)	Date (1981) / Day Number	4/13 103	5/11 131	6/8 159	6/29 180	7/13 194	7/27 208	8/10 221	8/24 236	9/8 251	9/21 264	10/5 278	10/26 299	11/19 323	12/14 348
	Larvae/m³	3	16	0	0.2	0.5	0.2	0.4	28	185	5.8	621	1645	547	0.1
	n measured	14	59		2	4	5	4	28	47	30	30	100	47	1
1	% <150	58	0		100	100	100	75	17	70	100	100	98	92	0
	% 150-200	21	0		0	0	0	0	83	28	0	0	2	8	0
	% >200	21	100		0	0	0	25	0	2	0	0	0	0	0
	Larvae/m³	0	74	0	0.4	0	170	71	320	270	129	2153	ns	272	52
	n measured		60		5		33	433	38	155	98	158		35	101
10	% <150		0		100		0	99	24	71	98	95		90	0
	% 150-200		0		0		9	0	76	15	0	4		8	0
	% >200		100		0		91	1	0	14	2	1		2	100
	Larvae/m³	5.7	2.2	0	0.3	0	1.2	677	330	3554	480	1843	ns	101	116
	n measured	19	11		4		6	30	30	90	128	56		46	272
20	% <150	94	0		100		0	100	60	0	18	89		96	0
	% 150-200	0	18		0		0	0	40	10	11	10		4	0
	% >200	6	82		0		100	0	0	90	71	1		0	100
	Larvae/m³	0.6	0.3	4.6	24	0.6	3.5	186	288	3025	639	2460	ns	311	83
	n measured	6	1	15	32	6	18	40	31	69	66	82		30	74
30	% <150	100	0	67	67	100	0	69	9	2	6	7		100	0
	% 150-200	0	0	27	33	0	17	26	65	12	12	20		0	16
	% >200	0	100	7	0	0	83	5	26	86	82	73		0	84
	Larvae/m³	0	1.5	0	9.3	0.7	26	7.4	69	416	109	4803	ns	59	0.3
	n measured		4		21	7	33	74	49	65	57	161		30	74
40	% <150		0		19	28	0	0	16	6	9	0		100	0
	% 150-200		0		57	44	0	20	14	29	0	0		0	0
	% >200		100		24	28	100	80	70	65	91	100		0	100

Bivalve Larvae

Few larvae were evident until early August when a large concentration was recorded at 20 m (Table 1). This larval concentration corresponded to both the 15°C isotherm (Figure 1) and a high chlorophyll *a* concentration (Figure 2). By contrast, low concentrations of larvae were recorded simultaneously in the

higher temperature (>19°C) surface water and at 40 m (Table 1). By early September very high (>3,000 larvae·m^{-3}) concentrations of larvae were recorded between the 17°C and 18°C isotherms at 20 to 30-m depth. Lower concentrations were again found at 1 to 10 m (<300 larvae·m^{-3}) and at 40 m (416 larvae·m^{-3}). Intense vertical mixing of the water column began by early October and uniformly high concentrations of larvae (>1800·m^{-3}) were recorded at 10 to 40-m depths with only slightly lower concentrations at the surface. High surface concentrations of larvae were evident until late November. Data for depths in the range of 10 to 40 m were unavailable in late October due to net failure; however, larval concentrations (59-272·m^{-3}) that were considerably below surface values (>547·m^{-3}) were recorded from 10 to 40 m in late November despite a well mixed, isothermal (10.5°C) water column. A decrease in water temperature in December coincided with lower larval concentrations (0.1-116·m^{-3} with greatest aggregation at 20 to 30 m) throughout the column.

Identification to the species level was attempted for 982 larvae; 922 of these were >200 μm in length (204 *Arctica islandica*(Linné), 207 *Spisula solidissima* (Dillwyn), 319 *Modiolus modiolus* (Linné), 89 *Anomia simplex* Orbigny, 163 unidentified). The remaining 60 larvae identified were *A. islandica* of approximately 110 μm length.

Only three larvae were identifiable to species from the samples collected on 13 April 1981 (day 103) and all were *S. solidissima* (Table 2). Although absolute numbers of larvae per tow collected on 11 May 1981 (day 131) were small; a large porportion of these were *A. islandica* at or approaching pediveliger stage of development (Table 2). These larvae were concentrated in the upper 20 m of the water column. A predominance of larvae >200 μm in length was not seen again until 27 July 1981 (day 208) when *S. solidissima* was evident between 10 and 40 m, including two pediveligers at 30 m, and *Modiolus modiolus* was present at 40 m. Larvae of *M. modiolus* were present in significant numbers in the >200 μm length

TABLE 2.

Species composition of bivalve larvae of >200 μm length collected at specific depths on the southern New England shelf during the period April-December 1981. (ns: no sample collected due to net failure.)

Depth (m)	Date 1981 / Day Number	4/13 103	5/11 131	6/8 159	6/29 180	7/13 194	7/27 208	8/10 221	8/24 236	9/8 251	9/21 264	10/5 278	10/26 299	11/19 323	12/14 348
	n identified	3	20	0	0	0	0	1	0	1	0	0	0	0	1
	Modiolus modiolus	0	0					0		1					0
1	*Spisula solidissima*	2	0					0		0					0
	Arctica islandica	0	20					0		0					0
	Anomia simplex	0	0					0		0					0
	Other	1	0					1		0					0
	n identified	0	60	0	0	0	30	1	0	14	2	0	ns	1	24
	Modiolus modiolus		0				1	0		9	0			0	24
10	*Spisula solidissima*		0				21	0		1	0			0	0
	Arctica islandica		57				0	0		0	0			1	0
	Anomia simplex		0				0	0		0				0	0
	Other		3				8	1		4	2			0	0
	n identified	1	11	0	0	0	6	0	0	82	73	0	ns	0	30
	Modiolus modiolus	0	0				0			45	20				30
20	*Spisula solidissima*	1	0				0			15	32				0
	Arctica islandica	0	9				0			7	4				0
	Anomia simplex	0	0				0			0	0				0
	Other	0	2				6			15	17				0
	n identified	0	1	1	0	0	5	30	32	61	76	48	ns	30*	16
	Modiolus modiolus		0	0			1	15	18	21	27	9			16
30	*Spisula solidissima*		0	0			2	0	1	12	31	22			0
	Arctica islandica		1	0			0	0	3	12	6	5		30*	0
	Anomia simplex		0	0			0	4	3	0	1	6			0
	Other		0	1			3	12	7	16	11	6			0
	n identified	0	4	0	5	1	33	41	15	74	45	70	ns	30*	3
	Modiolus modiolus		0		0	0	21	34	1	8	15	0			3
40	*Spisula solidissima*		0		0	0	2	3	5	40	17	0			0
	Arctica islandica		0		0	1	1	3	0	9	3	2		30*	0
	Anomia simplex		0		0	0	0	1	2	1	3	68			0
	Other		4		5	0	9	0	7	16	7	0			0

* Indicates identification of first shelled larvae at length of 110 μm.

fraction throughout August and September, and smaller numbers occurred in October. *Modiolus modiolus* was the only species present in the >200 μm length fraction in the depth range of 10 to 40 m in the samples collected on 14 December 1981 (day 348). Smaller (<200 μm length) larvae predominated throughout August-November at all depths except during the period 8 September to 5 October 1981 (days 251-278) in the depth range 20-40 m. In the >200 μm length range *S. solidissima* showed continuing presence during late August, September, and early October. Larvae of *A. islandica* of comparable size were recorded, usually at depths of ≥ 20 m, throughout August, September, and early October.

The data in Tables 1 and 2 can be combined to estimate absolute numbers of larvae per m^3 for each of the species listed in Table 2 for the length size range >200 μm. Highest concentrations of larvae of *S. solidissima* were recorded on 27 July 1981 (103 larvae·m^{-3} at 10 m), 8 September 1981 (585, 512, and 138 larvae·m^{-3} at 20, 30, and 40 m, respectively), 21 September 1981 (213 larvae·m^{-3} at 30 m), and 5 October 1981 (149 and 823 larvae·m^{-3} at 20 and 30 m, respectively). Highest concentrations of larvae of *M. modiolus* were recorded on 8 September 1981 (1,750 and 896 larvae·m^{-3} at 20 and 30 m, respectively), 21 September 1981 (93 and 186 larvae·m^{-3} at 20 and 30 m, respectively), and 5 October 1981 (337 larvae·m^{-3} at 30 m). Highest concentrations of larvae of *A. islandica* were recorded on 11 May 1981 (70 larvae·m^{-3} at 10 m), 8 September 1981 (273, 512, and 33 larvae·m^{-3} at 20, 30, and 40 m, respectively), and 5 October 1981 (187 and 138 larvae·m^{-3} at 30 and 40 m, respectively).

Identification of the smallest veliger stages (< 150 μm) was not generally attempted because of the large numbers present and the difficulty of making definitive identifications of such small larvae; however, the samples collected at 30 and 40 m during November were notable for the marked uniformity of the first shelled larvae present. Subsamples of 30 larvae per tow were examined and, based upon morphometry and measurements of length, height, and hinge length, they were identified as *A. islandica*. Larvae of *Anomia simplex* characterized by a notch in the ventral margin of one valve, were present throughout August, September, and October at depths in excess of 20 m.

DISCUSSION

The limited spatial and temporal extent of this survey and lack of sample replication clearly restrict interpretation of the resultant data. Only one station was sampled intensively in this study. Consequently, the contribution of horizontal, advective processes to temporal changes in physical structure of the water column, chlorophyll concentrations, and number of larvae collected cannot be definitively measured. On the other hand, the fact that adult stocks of the species denoted in Table 2 are widespread throughout the southern New England shelf and Middle Atlantic Bight (Merrill and Ropes 1969; Ropes 1978; Fogarty 1981; Theroux and Wigley 1983) and that the seasonal water temperature structure illustrated in Figure 1 is characteristic of the same area (Bigelow 1933; Ketchum and Corwin 1964; Beardsley and Boicourt 1981) suggests that larval data reported here may also be representative of that greater area.

In this study depth-specific plankton tows at intervals of 10 m were effected in preference to oblique tows predominantly because of equipment limitations. With such a protocol the possibility exists that significant numbers of larvae would not be representatively sampled because of aggregation caused by various environmental stimuli. Larval collections were made only between 1030 and 1430 hours. No examination of depths distribution were made at night. Mann (1986) reviews literature relating to phototactic behaviour in bivalve larvae. Only the data of Quayle (1952) for larvae of *Venerupis pullastra* (Montagu) provided any evidence of possible diurnal migration associated with changing light levels. (It was not possible to separate tidal and diurnal influences in Quayle's data.) Mann and Wolf (1983) reported that larvae of *Arctica islandica* exhibited little phototactic behaviour in laboratory systems. The larvae of *Modiolus modiolus* and *Spisula solidissima* have not been examined for phototactic behaviour. The influence of phototactically-induced, diurnal migration and aggregation on the distributions reported here cannot be definitively stated; however, the occurrence of larvae of *A. islandica*, *M. modiolus*, and *S. solidissima* over depth ranges greater than 10 m (see Table 2) argues against such aggregation.

The high concentrations of larvae recorded during late August and September were associated with water temperatures in the range of 14 to 18°C and decreasing chlorophyll content (Table 1; Figures 1 and 2). In contrast, the high concentration of larvae at the surface in October was associated with, but not necessarily related to, a higher chlorophyll *a* concentration that resulted predominantly from a bloom of the large diatom *Rhizosolenia* sp. It is relevant to ask whether or not chlorophyll concentrations are representative of food concentrations which are sufficient to sustain active feeding and growth of the larvae. Critical studies of bivalve larval feeding and growth have generally expressed food concentration in terms of cells·ml^{-1} (e.g., review by Epifanio 1976) rather than chlorophyll *a*. Chlorophyll *a* values in the field study varied from 0.1 to 3 μg·l^{-1}. Chlorophyll *a* to particulate organic carbon (POC) ratios have been widely reported and vary considerably (Steele and Baird 1961a, 1961b; Harris and Riley 1956; Goldman and Mann 1980). These values will, of course, include POC from sources other than cells that contain chlorophyll *a*. If within this range (1:23 - 1:260) a chlorophyll *a*: POC ratio of 100 is arbitrarily assumed, then a corresponding range of POC values over the time course of this study of 10 to 300 μg·l^{-1} is obtained. Goldman and Stanley (1974, Table 4) gave conversion factors for POC: cell concentration in the range 0.54 to 2.29 mg·l^{-1}: 10^5 cells·ml^{-1} depending upon the size of the algal cell. Application of maximum and minimum values to the present POC numbers resulted in a range of cell concentrations for the study period of 5.4 x 10^2 to 1.62 x 10^4 cells·ml^{-1} and 2.29 x 10^3 to

6.77 x 10^4 cells·ml^{-1}, respectively. Although grazing activity of molluscan veliger larvae decreases below 10^4 cells·ml^{-1} (Gallager and Mann 1980, Figure 3), significant grazing can still be effected at the lowest estimate of cell concentration. Furthermore, these values are probably more than adequate to sustain larval growth in that larvae of *A. islandica* grow well in food concentrations of 5 to 10 x 10^4 cells·ml^{-1} (Lutz et al. 1982) and larvae of *M. modiolus* grow well at 3 to 8 x 10^4 cells·ml^{-1} (de Schweinitz and Lutz 1976). With respect to the large bloom of *Rhizosolenia* sp. in October it is relevant to note that this species is probably too large to be ingested by most bivalve larvae and POC estimates that were obtained from chlorophyll *a* values at that time represent an over-estimation of food available to bivalve larvae.

The spawning season of *Modiolus modiolus* is poorly documented (de Schweinitz and Lutz 1976). The presence of larvae from July through December in the present study suggests that, on southern New England shelf at least, the spawning season for this species is protracted. Ropes (1968, 1978) and Jones (1981) both examined the spawning cycle of *Spisula solidissima* on the Middle Atlantic Shelf off New Jersey. Ropes (1968) reported two spawnings, a major event in July and August, and a minor event in October and November during the years 1962, 1963, and 1964. In 1965, by contrast, water temperatures were lower and only one spawning event, which was delayed and of longer duration than in 1962-64, was reported (Ropes 1968). Jones (1981) collected larvae of *S. solidissima* from a location inshore of the summer thermocline and reported a single spawning period that extended from June through November with a major concentration from August through October. Larvae of *S. solidissima* were present from late July through October in this study suggesting that, in the more northerly location, the spawning season may be terminated slightly earlier in the year.

Mann (1982) recorded a prolonged spawning season for *Arctica islandica* on the southern New England shelf from May through November with most intense spawning from August through November. Previously, Loosanoff (1953) reported a spawning season for *A. islandica* from the same location from June through mid October with maximum activity from August through early October. Further south, Jones (1981) described peak spawning as being "an autumnal to early winter event rather than summer/early autumn." Larvae of *A. islandica* were recorded on each sampling date from mid-July through early October as members of the size fraction that exceeded 200 μm in length. This observation is in agreement with the comments of Loosanoff (1953) and Mann (1982) on adult spawning periodicity. The occurrence of maximum numbers of >200 μm length larvae of *A. islandica* in September and October suggests that the period of maximum survival may be slightly more protracted than the period of October to November suggested in the hypothesis of Mann (1982). The large number of early shelled larvae (<150 μm length) of *A. islandica* recorded in mid-November probably arose from a spawning within the preceding two weeks based upon ambient seawater temperature (10.7°C) and laboratory growth-rate data (Lutz et al. 1982, Figure 2). Again, this indication of active spawning in late October to early November is in agreement with the comments of Mann (1982). The presence of these larvae suggests that the hypothesis of Mann (1982) is reasonable concerning the end of the period during which maximum survival occurs; unfortunately, the lack of data for late October that resulted from equipment failure does not allow further statements on the last date of occurrence of large (>200 μm) larvae of *A. islandica* in the water column.

An explanation of the presence of larvae of *A. islandica* of length >200 μm in May is problematic. At the ambient temperature on the day of sample collection (7.3°C at 0 m increasing to 9.5°C at 43 m) growth to >200 μm length would probably take 6 weeks or longer (Lutz et al. 1982, Fig. 2), especially considering the fact that temperature was increasing at that time. The earliest that those larvae could have been spawned would, therefore, be late March-April, a period when water temperatures reach an annual low of ~1°C in this region and when very little spawning activity was noted by either Loosanoff (1953) or Mann (1982). The possibility exists that these larvae may have originated from a late autumn spawning during the preceding spawning season. This possibility is supported by the presence of large numbers of early veliger larvare of *A. islandica* in samples collected in November (Table 2); however, survival of larvae through the winter months would also necessitate a planktonic life of approximately 6 months, an ability to survive essentially arrested growth periods at low temperatures, and the good fortune not to be either lost to predation or washed out of a region in which recruitment may occur. A study of the effect of extended periods of low temperature on the development of larvae of *A. islandica* is required to evaluate the potential contribution of overwintering larvae to maintenance of this species on the New England shelf.

Franz and Merrill (1980) reported the bathymetric range of adults of *A. islandica* and *M. modiolus* in the Middle Atlantic Bight as 9 to 165 m and 1 to 146 m, respectively. Theroux and Wigley (1983) reported that *M. modiolus* occupies a bathymetric range of 13 to 256 m; the majority of their samples were taken between 25 and 49 m. Both *A. islandica* and *M. modiolus*, therefore, exhibit submergence south of Cape Cod; however, Mann and Wolf (1983) noted that in more northerly parts of its zoogeographic range *A. islandica* can be found in shallow, sublittoral depths. *Modiolus modiolus* also occurs in shallower depths in more northerly locations. Gosner (1971) reported that *M. modiolus* occurs from slightly below tide level to 81 m, whereas de Schweinitz and Lutz (1976) noted that the species can be "collected subtidally along the coast of Chamberlain, Maine." Cragg (1980) reported that selection favours a more sensitive depth regulation mechanism in the larvae of littoral species than in the larvae of sublittoral species. If this regulation translates as comparable depth regulation in species that occupy similar bathymetric ranges, then larvae of *A. islandica* and *M. modiolus* would be expected to control their depth within and occupy similar bathymetric ranges. Although this aspect

of the larval biology of *M. modiolus* has not been examined experimentally in the laboratory, the similarity of depth distribution of larvae in the field (Table 2) is notable.

Although *S. solidissima* occupies a shallower bathymetric range (Merrill and Ropes 1969) than *A. islandica* or *M. modiolus*, Franz and Merrill (1980) considered it a member of the boreal fauna. Theroux and Wigley (1983) stated that *S. solidissima* "inhabits the Boreal, Virginian and Carolinean provinces in the northwest Atlantic" and occupies "primarily inshore shallow waters." The depth regulation of larvae of *S. solidissima* have not been examined; however, these larvae are more tolerant of higher temperatures, reaching metamorphosis in 19 days at 22°C (Loosanoff and Davis 1963), than either *M. modiolus* (16-21.5°C, de Schweinitz and Lutz 1976) or *A. islandica* (< 15°C, Landers 1976; Lutz et al. 1982). The larval development temperature of 22°C for *S. solidissima* is compatible with both the observation of larvae of *S. solidissima* at 20.3°C and 10 m depth on day 208 of this study and the temperature of the overlaying water during the period of spawning of adults of *S. solidissima* in the Middle Atlantic Bight (see Ropes [1968, 1978] and Jones [1981] for data on spawning; and Bigelow [1933] for water temperature data).

The southern range extension of such boreal species as *A. islandica* and *M. modiolus* on the shallow continental shelf south of Cape Cod appears to be made possible by the presence of the summer "cold pool" below the depth of the seasonal thermocline (compare zoogeographical data of Theroux and Wigley [1983] with the physical data of Bigelow [1933]). It would clearly be profitable to examine the seasonal occurrence and survival to metamorphic size of the larvae of these boreal species in the water column of the southern New England shelf and Middle Atlantic Bight over a number of years. This option is particularly attractive in examining recruitment in *A. islandica*, a long lived species (Thompson et al. 1980) which, despite apparent regular spawning activity (Loosanoff 1953; Jones 1981; Mann 1982), exhibits a lack of representation in small (< 20 mm longest dimension) size classes in the Rhode Island Sound region (Mann, unpublished data collected 1978-1981). The relative contributions of lack of recruitment and predation (see Franz and Worley [1982] for comments on predation on juveniles of *A. islandica*) to this observation have yet to be defined. This approach might also provide insight into the recruitment of *S. solidissima* on the southern shore of Long Island, NY, a process which according to Franz (1976) is "apparently dependent on massive settlements of larvae occurring irregularly and infrequently."

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RESPONSES OF THE HARD CLAM *MERCENARIA MERCENARIA* (LINNÉ) TO INDUCTION OF SPAWNING BY SEROTONIN[1]

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ABSTRACT Clam size, sex of clam, concentration of serotonin, and site of administration of serotonin were found to influence the induction of spawning in the hard clam *Mercenaria mercenaria* (Linné). Overall, male clams greater than 36.4 mm thickness were more likely to spawn in response to serotonin injection at concentrations of 0.2 or 2.0-mM. Administration of serotonin by injection in the anterior adductor muscle resulted in significantly more spawnings than intragonadal injection or dispersal in water surrounding the incurrent siphon.

KEY WORDS: hard clam, *Mercenaria mercenaria*, spawning, serotonin

INTRODUCTION

The chemical serotonin or 5-hydroxytryptamine (5-HT) has been shown to be an effective inducer of spawning for many bivalves (Matsutani and Nomura 1982; Gibbons et al. 1983; Gibbons and Castagna 1984). Injection of serotonin into the gonad or adductor muscle of ripe bivalves will induce spawning without any additional stimuli; however, male bivalves spawn in greater numbers than females. This study examines the effectiveness of various concentrations of serotonin to induce spawning in male and female hard clams *Mercenaria mercenaria* (Linné) of three adult size classes. Different sites of administration of serotonin were also investigated.

MATERIALS AND METHODS

The method of individual spawning described by Castagna and Kraeuter (1981) was used to spawn all hard clams without any thermal shock or additional stimuli. Hard clams were spawned by placing individuals in glass dishes containing 1 *l* of 1-μm-filtered seawater (30 ppt) in water baths at 20°C. Crystalline serotonin (5-hydroxytryptamine, creatinine sulfate complex, Sigma Chemical Company, St. Louis, MO) was dissolved in 1-μm-filtered seawater to prepare the required concentrations of serotonin solutions.

A determination of ripeness and sex of hard clams was made by drilling a small hole through the shell and removing a biopsy of the gonad for microscopic examination (Castagna and Kraeuter 1981). Gravid hard clams were divided into six groups of 150 individuals by size and sex. Hard clams were classified by size as littlenecks (25.4-36.4 mm in thickness), cherrystones (> 36.4-41.2 mm), and chowders (> 41.2 mm). Small notches were filed into the valve margins of clams adjacent to the anterior adductor muscle. Hypodermic injections of 0.4 ml of 0 (control), 0.02, 0.2, 2.0, or 20.0-mM serotonin solutions were made into anterior adductor muscles. Each concentration of serotonin was given to 30 hard clams of each sex-size class. Observations were made of the time interval to spawning and the behavior of clams after injections. A three-way analysis of variance was used, after log (X+1) transformation of data, to test the effects of clam size, sex, and concentration of serotonin on numbers of hard clams induced to spawn (Sokal and Rohlf 1981).

The influence of administration site on the efficacy of serotonin to induce spawning was examined using 675 cherrystone clams. Three sites were tested using 225 clams each. The two groups received 0.4 ml of 2.0-mM serotonin solution by injection. One group of clams had notches filed into the valve margins adjacent to the anterior adductor muscle which allowed injection of serotonin into the muscle. The second group was injected intragonadally by inserting a needle between the valves immediately below the ligament on the posterior end of the clam. A new needle was used for each clam to prevent transference of gonadal products. The third group received 0.4 ml of 20.0-mM serotonin dispersed into the water entering the incurrent siphon. The efficacy of the various sites of serotonin administration upon induction of spawning was examined through the nonparametric Kruskal-Wallis test (Sokal and Rohlf 1981).

The possibility of using the foot as a site of serotonin injection was investigated using 380 male cherrystone and chowder clams. Small notches were filed into the lip of hard clam valves. Half of the clams were injected in the foot with 0.4 ml of 2.0-mM serotonin solution while the control group received 0.4 ml of 1-μm-filtered seawater. The G-test of independence and William's correction were used to test whether spawning was independent of serotonin injection into the foot (Sokal and Rohlf 1981).

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RESULTS

Hard clams reacted immediately to injection with serotonin into the anterior adductor muscle, gonad, and foot by increased siphoning, probing with an elongated foot, and adduction of the valves. Injection of serotonin in ripe hard clams induced spawning within 10-15 minutes and the majority spawned within 30-60 minutes. Control animals did not exhibit any of these behaviors and did not spawn. No mortality occurred in the 1,955 hard clams used in these experiments.

Sex of clams, size of clams, and concentration of serotonin were found to significantly influence ($P < 0.005$) the induction of spawning. Overall, male clams were seven times more likely to spawn than females upon injection with serotonin into the anterior adductor muscle (Table 1). For male hard clams, chowders and cherrystones were more likely to respond to serotonin injection by spawning than littlenecks. More specifically, male cherrystones (40.0%, 60.0%) and chowders (66.7%, 53.3%) spawned in significantly greater numbers ($P < 0.001$) at 0.2 and 2.0-mM serotonin solutions, respectively, than male littlenecks and female clams of all three size classes at any serotonin concentration. Of the female hard clams, chowders (23.3%) injected with 0.2-mM serotonin solution were the most likely to respond by spawning.

Occasionally both male and female hard clams would react to serotonin injections at concentrations of 0.2 to 20.0-mM by closing the valves on the end of the foot, often cutting the tissues (Table 2). This behavior is referred to as foot nipping. The effect of concentration was significant ($P < 0.001$), as greater numbers of clams displayed foot nipping at higher concentrations. Chowders of both sexes had greater tendencies ($P < 0.01$) to display this behavior than littlenecks or cherrystones. There was no significant statistical difference between the numbers of clams of either sex that showed foot nipping.

Injection of 2.0-mM serotonin solution into the anterior adductor muscle of the hard clam induced significantly more ($P < 0.001$) spawning than intragonadal injection or dispersal in water surrounding incurrent siphons (Table 3). Release of serotonin into seawater did not cause hard clams to display any behavioral responses to the serotonin or to spawn.

Injection of 2.0-mM serotonin into the foot of the hard clam induced significant numbers ($P < 0.001$) to spawn ($N = 86$). None of the control animals spawned. Hard clams reacted to insertion of hypodermic needles by withdrawing the foot.

TABLE 1.

Numbers of males and females of *Mercenaria mercenaria* of three size classes induced to spawn by injection of serotonin at five concentrations (N = 30 clams tested for each size class and concentration).

		Concentrations of Serotonin (mM)					
Sex	Size Class	0	0.02	0.2	2.0	20.0	Total
Male	Littleneck	0	3	1	2	0	6
	Cherrystone	0	6	12	18	12	48
	Chowder	0	4	20	16	9	49
Female	Littleneck	0	1	1	0	0	2
	Cherrystone	0	1	3	0	0	4
	Chowder	0	0	7	1	0	8
Total Number Males		0	13	33	36	21	103
Total Number Females		0	2	11	1	0	14
Percentage of Males		0	14.4	36.6	40.0	23.3	22.9
Percentage of Females		0	2.2	12.2	1.1	0	3.1

TABLE 2.

Numbers of males and females of *Mercenaria mercenaria* of three size classes responding to serotonin injection at five concentrations by nipping off the end of the foot (N = 30 clams tested for each size class and concentration).

		Concentrations of Serotonin (mM)					
Sex	Size Class	0	0.02	0.2	2.0	20.0	Total
Male	Littleneck	0	0	0	0	1	1
	Cherrystone	0	0	1	2	7	10
	Chowder	0	0	0	1	11	12
Female	Littleneck	0	0	1	0	3	4
	Cherrystone	0	0	0	1	6	7
	Chowder	0	0	1	4	20	25
Total Number Males		0	0	1	3	19	23
Total Number Females		0	0	2	5	29	36
Percentage of Males		0	0	2.2	3.3	21.1	5.1
Percentage of Females		0	0	1.1	5.6	32.2	8.0

TABLE 3.

Numbers of hard clams (*Mercenaria mercenaria*) induced to spawn by administration of serotonin by injection into the anterior adductor mucle, intragonadal injection, and dispersal in water surrounding the incurrent siphon (N = 25 clams tested for each site and replicate).

	Site of Serotonin Administration		
	Anterior Adductor Muscle	Gonad	Seawater
	6	5	0
	8	2	0
	9	6	0
	5	4	0
	7	1	0
	5	5	0
	5	4	0
	7	10	0
	9	4	0
Total number	61	41	0
Percentage	27.1	18.2	0

DISCUSSION

Serotonin, a molluscan neurotransmitter, occurs naturally in the visceral ganglia of *Mercenaria mercenaria* at mean concentrations of 40 $\mu g \cdot g^{-1}$ of fresh tissue (Welsh and Moorhead 1960). In the hard clam, laboratory studies have shown that serotonin increases the amplitude of the beating of isolated hearts, stimulates the beating of lateral cilia on the gill, and increases

the tone and induces rhythmic activity in the rectal muscle (Welsh 1957; Aiello 1970; Leake and Walker 1980). The role of serotonin in the spawning of bivalves is unknown.

Concentrations of 0.2 or 2.0-mM serotonin appeared to be most effective to induce ripe hard clams to spawn. Serotonin induced primarily male clams to spawn but similar numbers of males and females displayed foot nipping when exposed to high serotonin concentrations. Matsutani and Nomura (1982) found that intragonadal injection of ganglionic homogenates of the dioecious scallop *Patinopecten yessoensis* (Jay) induced spawning only in males. Injection of serotonin into the gonads of scallops induced spawning in both males and females, but males spawned more quickly and at lower serotonin concentrations.

Injection of serotonin solutions into the gonad or a blood sinus such as found in the anterior adductor muscle or foot induced spawning in ripe hard clams. The anterior adductor muscle proved to be a convenient and effective site for injection of serotonin. The shell margins adjacent to the muscle are easily notched with a file and the notch is repairable by the clam. A needle can be inserted directly into the anterior adductor muscle with certainty of destination as the resistance of the muscle is easily felt. Injection of serotonin solutions into the gonad or foot required a longer hypodermic needle. Hard clams reacted to insertion of the needle into the foot by withdrawing it. For intragonadal injection the needle must pass through overlying tissues when inserting it through a hole drilled in the shell over the gonad or between the valves on the posterior side of the ligament. Care must be taken to insert the needle into the gonad and not other surrounding organs.

Serotonin may be used to induce rapid and synchronous spawning of the hard clam. This technique may be easily applied to individual and mass spawning techniques. Since there is no need to heat seawater to thermally shock clams to spawn, bacterial growth in the culture of eggs may be reduced. Serotonin may also be used to induce spawning in other bivalves, such as the ocean quahog *Arctica islandica* (Linné), which are resistant to traditional spawning stimuli (Gibbons et al. 1983). This technique offers another way to selectively breed broodstocks for improvement or genetic research.

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THE EFFECTS OF SEED SIZE, SHELL BAGS, CRAB TRAPS, AND NETTING ON THE SURVIVAL OF THE NORTHERN HARD CLAM *MERCENARIA MERCENARIA* (LINNÉ)[3]

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ABSTRACT Seed size at planting is the dominant factor affecting hard clam survival to marketable size when field grow-out techniques are used. The use of plastic mesh nets, crab traps, and wire mesh bags (filled with oyster shells) alone or in combination can be used to increase survival of hard clams of ≥6 to 8-mm shell height. These techniques do not provide sufficient protection for 2-mm seed. The combination of net + crab trap + shell bag was nearly twice as effective as the net alone when 10 to 14-mm seed was used and over five times as effective as the net alone when 6 to 8-mm seed were planted. Survival in excess of 50% slows the growth rate and yields higher percentages of submarketable, <25-mm thick (New York legal limit) clams. Local markets and dealers would accept all clams >22 mm.

KEY WORDS: Hard clam, *Mercenaria mercenaia*, survival, predator exclusion.

INTRODUCTION

Commercial planting of seed clams for field growth to marketable size requires a series of decisions based on: size and cost of clam seed, cost of providing protection, the specific environment, and the predators that are present. When small seed clams are first planted, smaller predators may destroy a significant portion of the seed (Castagna and Kraeuter 1977, 1981; Eldridge et al. 1979). Experiments have shown that survival of clams in field plots is dependent on the presence of adequate protection throughout the warmer months (April-October) until clams are harvested (Kraeuter and Castagna 1980). The present series of tests were designed to examine the effects of predator protection provided by nets, shell bags, and crab traps to a size series of hard clam seed. Nets were considered to be useful in preventing clam seed predation by the blue crab *Callinectes sapidus* Rathbun and as the clams neared harvest size, predation by the cow-nosed rays *Rhinoptera bonasus* (Mitchill) (Kraeuter and Castagna 1980). Crab traps were used in an attempt to reduce predation by blue crabs, and shell bags were used in an attempt to trap xanthid crabs, chiefly *Panopeus herbstii* H. Milne-Edwards and *Neopanope texana* (Smith). These latter species are not attracted to baits, but are cryptic by nature and are found hiding in shell debris or among clumps of oysters. The bag of oyster shells provided a habitat which could readily be removed along with the crabs. The tested hypothesis was that combinations of these protective devices should increase survival of the hard clam *Mercenaria mercenaria* if these predator species significantly affected clam survival. This is the first in a series of experiments designed to test the effectiveness of various protection methods and the interactions between clam size and those techniques.

[3]Contribution No. 1387 from Virginia Institute of Marine Science, The College of William and Mary, Gloucester Point, Virginia.

MATERIALS AND METHODS

All experiments were conducted in Bradfords Bay near the town of Wachapreague, VA. Bradfords Bay is typical of the circular or nearly circular lagoonal bays of the ocean side of Virginia's Eastern Shore. This marsh-lagoon complex consists of shallow bays with extensive mudflats and oyster reefs surrounded by a salt marsh that is dominated by *Spartina alterniflora* Loisel. A minimum of freshwater flows into the system and salinities remain high throughout most of the year. Substrates are typically sandy behind barrier islands and near ocean inlets, and become progressively muddier toward the mainland. The experimental site was on muddy substrate, in the intertidal zone, and outside a fringe of oyster reefs. Water temperatures at the site ranged from -1 to 30°C and salinities ranged from 14 to 33 $^{0}/_{00}$.

Seed clams that were used during the experiments were reared in the culture facility of Virginia Institute of Marine Science from spawns conducted the preceding year. These clams were over wintered in flowing seawater tables and graded by size in the spring. The rearing and grading techniques have been described (Castagna and Kraeuter 1981). Replicate plots were prepared by spreading gravel about 3 cm thick on the mud substrate one week prior to planting the clams (Castagna and Kraeuter 1981; Kraeuter and Castagna 1977). Four gravel plots were laid out with at least 30 m between them in separate parts of the intertidal zone. Plots 1 and 4 contained five experimental treatment sites 1.5 x 1.5 m (5 x 5 ft) in size and Plots 2 and 3 were similar except only four treatment areas were constructed. The size of the experimental treatment area was chosen to make these experiments directly comparable to our previous studies (Kraeuter and Castagna 1977, 1980). The treatment areas were designed to test survival of three sizes of clam seed with various combinations of shell bags, crab traps, and nets (Table 1).

Clams were graded into three size categories 2 mm, 6 to 8 mm, and 10 to 14 mm, and groups of 8,000 2-mm, 6,000 6 to 8-mm, and 5,475 10 to 14-mm clams were placed in mesh bags for transport to the field and planted in the appropriate plots (Table 1). These replicate lots of seed clams were randomly assigned to a particular plot and planted at low tide when the plots were exposed. All clams were planted in May 1979.

Nets made of 12.5-mm mesh, Conwed® plastic, were stretched loosely over the gravel substrate. Edges of the nets were embedded in the mud and held in place with steel reinforcing rods placed over the mesh and pressed into the mud. Crab traps of standard commercial design were baited with fish and emptied every two days during warm months when blue crabs were active. Shell bags (45 cm wide x 60 cm long) were constructed of hexagonal wire mesh with 3.8-cm openings and filled with oyster shell and sealed. These bags were placed on the bottom near the appropriate plot and allowed to remain for 1 wk. Bags were removed from the water by pulling them quickly on board to prevent resident crabs from escaping. Each was then replaced with a new shell bag. Shell bags were removed during the colder months when crabs were no longer active. During the second year the bags were replaced every 2 wks. Four crab pots and six shell bags were located around the periphery of the appropriate plot. Experimental control areas with gravel but without nets were established for each trial. Full series of treatments were established for the two smaller seed sizes, but the 10 to 14-mm clams were not sufficiently abundant to conduct a full sequence of tests. We selected treatments that represented two ends of the spectrum for these larger clams (Table 1). More clams were planted in the 2-mm size class than in the 6 to 8-mm class because previous studies had shown that survival was seed-size dependent.

All plots were harvested in their entirety during October 1980. The cumulative effects of all factors are best depicted by harvest data (Kraeuter and Castagna 1980). Comparisons between treatments are analyzed by Chi-square tests following the methods outlined in Snedecor (1962).

TABLE 1.

Experimental design of plots. Numbers indicate the number of clams planted in each 1.5 x 1.5-m plot. All clam sizes are based on square mesh size required to retain the clams.

Plot		Number of Seed Clams and Sizes (mm)		
		2	6 to 8	10 to 14
	Treatment			
1	Net	8,000	6,000	5,475
	Control	8,000	6,000	
2	Net + Shell Bags	8,000	6,000	
	Shell Bags	8,000	6,000	
3	Net + Crab Traps	8,000	6,000	
	Crab Traps	8,000	6,000	
4	Net + Shell Bags + Crab Traps	8,000	6,000	5,475
	Shell Bags + Crab Traps	8,000	6,000	

RESULTS

Previous studies have shown that seed size at planting is a dominant factor in determining the survival to harvest, and our present data provide further confirmation, (X^2 = 59,942, df 2). Seed size was dominant, therefore, the remaining analyses were carried out within a seed-size category. Our basic assumption was that there should be no difference between treatments within a seed-size class.

Significant differences were found between treatments for 2-mm seed. These seed had significantly greater survival than expected in the Shell Bag + Trap (BP) series (Table 2). This

TABLE 2.

Numbers of hard clams harvested (by treatment). Chi-square values are based on analyses within a planted size.

Treatment*	Planted Size					
	2 mm		6 to 8 mm		10 to 14 mm	
	Number	X^2**	Number	X^2**	Number	X^2**
C	7	5.6 L***	15	225.7 L***		
T	21	1.0	284	6.2 G	2627	862 L
B	5	8.2 L	3	249.8 L		
TB	16	0.03	73	126.5 L		
P	5	8.2 L	1	254.0 L		
TP	10	2.7	46	169.1 L		
BP	50	66.1 G	4	247.7 L		
TBP	20	0.6	1539	7101.1 G	4676	862 G
		92.7**		8380.1**		1724**
df		7		7		1

* Treatments: C = Control, T = Net, B = Shell Bag, P = Crab Trap, and combinations thereof.

** Chi-square = 3.84 @ 95% (1 df) and 6.63 @ 99% (1 df).
Chi-square = 14.07 @ 95% (7 df) and 18.48 @ 99% (7 df).

*** G = greater survival than expected.
L = less survival than expected.

TABLE 3.

Numbers of clams harvested, percent survival (%), and mean size ($\bar{X}$) in mm of harvested experimental plots by treatment.

Treatment*	Planted Size								
	2 mm			6 to 8 mm			10 to 14 mm		
	Number	%	$\bar{X}$	Number	%	$\bar{X}$	Number	%	$\bar{X}$
C	7	< 0.1	26	15	0.2	31			
T	21	0.2	41	284	4.7	39	2,627	47.9	36
B	5	0.1	38	3	< 0.1	35			
T B	16	0.2	36	73	1.2	40			
P	5	< 0.1	41	1	< 0.1	55			
T P	10	0.1	44	46	0.7	42			
B P	50	0.6	34	4	< 0.1	35			
T B P	20	0.2	38	1,539	25.6	38	4,676	85.4	30

* Treatments: C = Control, T = Net, B = Shell Bag, P = Crab Trap, and combinations thereof.

anomaly resulted from an experimental artifact. During the experiment, winter storms caused a rip in the net and clams from an adjacent bed of 10 to 14-mm seed were washed into the 2-mm BP plot. This small washover was enough to cause statistically significant results because survival was so poor in plots with 2-mm seed (all less than 1%). The interpretation of the usefulness of these protection methods for commercial plantings of 2-mm and other sizes was not affected because the total number of clams that moved was relatively small.

Results for the 6 to 8-mm seed were as expected with the interaction of nets with bags and crab traps providing highly significant increases in survival (Table 2). All other tests yielded less than expected survival with the somewhat surprising exception that greater than expected survival resulted when neither shell bags nor crab traps were present. It may be that the presence of either the bag or the traps alone attracted both blue crabs and xanthid crabs so that without the means of removing these alternative predators they were able to take advantage of the situation. It is also possible that the result may be a chance occurrence. A third possibility is that washover similar to that of the 10 to 14-mm seed into the 2-mm seed plot may have occurred, but we do not have direct evidence for washover in this instance.

Ten to 14-mm seed clams survived well in both the Net (T) and Net + Shell Bag + Crab Trap (TBP) treatments (Table 2), but significantly more survived when all three treatments were combined. Total survival for the combined treatments (BP = 85%) was nearly double that of the net alone (T = 48%) (Table 3).

Growth data indicated a rapid increase in size during the first summer and some individuals in the 6 to 8-mm clam plot reached nearly the same size as their counterparts in the 10 to 14-mm clam plots. When the experiment was terminated, the mean size data indicated that those plots with greatest survival had reduced growth rates because of crowding (Table 3).

DISCUSSION

Only three of the experimental treatments provided survival that was at or near commercially acceptable levels. The Net + Shell Bag + Crab Trap (TBP) combination was more effective than the net alone, and in 6 to 8-mm seed plot, survival was over five times greater with all three protective devices present. This is similar to our previous experiments (Castagna and Kraeuter 1977; Kraeuter and Castagna 1977, 1980) where interactions were not additive and all combinations were necessary for maximum survival. The importance of size at planting cannot be overlooked. This is emphasized because, although there were significant statistical differences, none of the combinations was able to produce survival in excess of 1% for the 2-mm seed clams. These survival data extend our previous findings to smaller seed sizes and emphasize the need for multiple protective devices to ensure that large numbers of clams survive. The data presented by Flagg and Malouf (1983) for hard-clam plantings in New York support our results and indicate the need

TABLE 4.

Numbers of clams in various marketable categories.

Plot*	Size at Planting in mm	Size at Harvest (in mm)					
		>25	25 to 24	<24 to 22	<22	Total	Marketable
T B P	6 to 8	496	324	388	331	1,539	1,208
T B P	10 to 14	545	398	601	3,132	4,676	1,544
T	10 to 14	763	551**	656**	639*	2,600*	1,970*

* Plots: T = Net Cover, TBP = Net Cover + Shell Bag + Crab Trap.

** Calculated values

for multiple protective devices in other locations.

Clams from two of the three plots that yielded the greatest survival were graded (by width [thickness]) to determine marketability. All clams that were >22-mm thick were acceptable to the local market and dealers. In spite of the stunting that was evident in the bed with the greatest survival (30.5 ± 1.08-mm; mean size ± standard error t = 0.05), greater numbers of marketable clams were available in all categories than in the bed with larger mean size (37.9 ± 1.07 mm) (Table 4). The harvest from the plot that was protected by a net with initial 10 to 14-mm seed clams (2,627 survivors) was not graded except to New York market standards of 1 inch (25 mm) thickness. Twenty-nine percent of these clams were >25 mm suggesting growth similar to the 6 to 8-mm seed treatment with 1,539 survivors. The data do not indicate significant mean size differences (36.8 ± 1.04 mm *vs* 37.9 ± 1.07). Based on these data, we would expect percentage breakdown of the remaining clam size-classes to be equivalent to the data from the 6 to 8-mm TBP plot and thus a total of 1,970 individuals would be marketable. A portion (3,272) of the nonmarketable clams were replanted to be harvested within 1 year. The decision of whether to thin the beds after the first year or to wait for harvest and then to replant is based on economics and the additional growth rates. Studies of these interactions are currently underway.

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HISTOMORPHOLOGICAL ASPECTS OF THE GONADS OF *CHAMELEA GALLINA* (LINNÉ) (BIVALVIA; VENERIDAE) IN AUTUMN

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ABSTRACT The gametogenetic activity and gonadal renewal of *Chamelea gallina* from Cesenatico (Adriatic Sea) was examined in the period September-December 1982, after the massive summer spawning. Histomorphological aspects of the reproductive cycle are described. Rudimentary hermaphroditism was observed in some specimens. In autumn both gamete emission and gonadal renewal occur.

KEY WORDS: Gametogenesis, spawning, acinus renewal, hermaphroditism

INTRODUCTION

The biological cycle of the Chicken Venus clam *Chamelea gallina* (Linné) (syn. *Venus gallina* Linné) of the Adriatic Sea has been examined by several researchers (Salvatorelli 1967; Poggiani et al. 1973; Froglia 1975; Marano et al. 1980). Previous observations allowed description of the spawning cycle of the species and the promotion of low-catch limitations in order to protect the most critical phases of its growth cycle from fishery pressures. There are, however, some discordant or, at least, incomplete data concerning the gametogenetic cycle phases after the massive summer spawning regarding the onset and the presence of a possible second reproductive period in the autumn (Guerin 1973; Froglia 1975).

According to Salvatorelli (1967) and Poggiani et al. (1973) gametogenesis again occurs after a prolonged restoration period. According to Marano et al. (1980) gametogenesis seems to be almost constant after the massive summer emission, with a slow beginning in September- October. For these reasons we considered it useful to further examine the relative gonadal growth of *C. gallina* to qualify in detail the histological stages and phases occurring in the period following the massive summer spawning, that is to say, from September to December.

MATERIALS AND METHODS

The specimens of *C. gallina* came from the marine biological station indicated by No. 14 off Cesenatico, in water 1.5-2 m deep, 100-150 m offshore. The hydrological data relative to the four samples examined were supplied by the "Study Center" of Cesenatico (Table 1).

The population of *Chamelea gallina* was sampled during September-December by means of a clam rake which could select specimens longer than 20mm and, therefore, sexually mature. From each sample a subsample of 50 specimens was examined. The shell length (SL) was used as an indicative measure of age. Individuals were fixed in Bouin's fluid, embedded in paraffin for sectioning (5 -10 μm), and stained with Mayer's acid haemalum-eosin.

TABLE 1.

Hydrological data relative to Station 14 in the period September-December 1982 (Cesenatico, Adriatic Sea).

Sample Dates (1982)	Sample depth (cm)	Temperature (°C)	Salinity (‰)	Dissolved Oxygen (mg·l^{-1})	Chlorophyll "a" (μg·l^{-1})
9 September	55	20.4	33.7	6.6	6.9
8 October	55	20.4	36.8	3.0	2.4
19 November	55	10.8	25.2	7.4	3.6
15 December	55	9.5	29.0	9.4	3.2

Gonadal development was staged according to a modification of the histological classification of Tranter as reported by Lucas (1965; pages 138, 139). The Tranter classification includes five stages of female and male gonadal growth (Fd_1 -→ Fd_5; Md_1 -→ Md_5) plus three stages of regression (Fr_1 -→ Fr_3; Mr_1 -→ Mr_3). The same gonad shows particular characteristics at several stages either due to a differential growth of alveoli (e.g., Md_5 + Mr_1) or to the overlapping of different phases in the same alveolus (e.g., Mr_3/Md_1).

In our work the stages indicated as Fr_1, Fr_2, Fr_3, and Mr_1, Mr_2, Mr_3 are qualified as:

Fr_1, Mr_1 - gamete emission (partial or almost total);
Fr_2, Mr_2 - acinus cleaning (often phagocyte presence);
Fr_3, Mr_3 - presence of a vacuolated tissue penetrating the alveolus.

Regression stages are sexually recognizable only if residual gametes are present or if they are associated with active alveoli. We characterized as Fr_3 (or Mr_3) simple stage those gonads in which this phase is clearly prevalent.

The percentage frequency of the different stages were calculated on the total of the females and males examined

monthly, excluding hermaphroditic and undeterminable specimens (Table 2 and Figs. 1, 2).

RESULTS AND DISCUSSION

In the examined specimens both female and male gonads consist of alveoli and extend under the heart, between the body musclar wall and the stomach, and beneath the visceral sac over the foot. They show, however, differential stage growth in different individuals. The histological examination carried out on the specimens, both characterized for sex and gonadal growth, brought us to identification of the stages reported below.

Female Gonadal Growth

Gonadal growth in the females may be characterized by the following main stages grouped in three principal phases (Table 2, Fig. 1):

1. gametogenesis onset: Fd_1, $Fd_1 + Fd_2$, $Fd_1 + Fd_2 + Fd_3$ (or $\dashrightarrow Fd_2 + Fd_3$);
2. gamete emission: Fd_5/Fr_1, $Fr_1 + Fd_1 + Fd_2$;
3. acinus renewal: Fr_2, $Fr_2 + Fr_3$, Fr_3, $Fr_3 + Fd_1$.

The onset of gametogenesis clearly occurs in October. Fd_1 is the initial oogenesis stage; the alveolus wall is filled by vacuolated tissue. In all of the September and some of the October specimens the gonads are almost atrophic and growth can be classified as the composite stage $Fr_3 + Fd_1$; the alveolus wall is unbroken but very thin. Protogonia (10 x 12 μm), oogonia (5-6 μm, diam), and young oocytes (15-20 μm) are present at the periphery; they are apparently intact but faintly stained. The vacuolated tissue, too, is thin and not compact. From October onwards the Fd_1 stage shows more numerous, clearly basophile germinal cells and from November they are often engaged in mitosis (Figs. 3, 4). The vacuolated tissue that penetrates the alveolus cavity is generally compact. From November this stage is always found together with advanced stages: $Fd_1 + Fd_2$, $Fd_1 + Fd_2 + Fd_3$ (or $\dashrightarrow Fd_2 + Fd_3$) (Figs. 5, 6). The most mature alveoli show oocytes at the onset of vitellogenesis ($\leqslant 60$ x 40 μm) (Fig. 5).

Gamete emission occurs in September-October specimens. Egg spawning is characterized by the Fd_5/Fr_1 stage and can be either partial or total in different alveoli of the same ovary. Rare oogonia are present on the acinus wall (Fig. 7). Other specimens ($Fr_1 + Fd_1 + Fd_2$) show gonads in which some alveoli have already discharged (Fr_1) and alveoli at the onset of gametogenesis ($Fd_1 + Fd_2$).

Acinus renewal occurs in September-October specimens. Alveolus cleaning is characterized by the Fr_2 stage, and is often associated with the presence of phagocytes (Fig. 8). In the alveolus wall, however, protogonia are still intermingled with somatic cells. Acinus renewal is further characterized by composite stages $Fr_2 + Fr_3$ and $Fr_3 + Fd_1$ (gametogenesis onset). At the Fr_3 stage a vacuolated tissue fills the alveolus cavity; protogonia and gonia are present at the periphery (Fig. 10).

In September some specimens were sexually undeterminable. Some protogonia persisted (Fig. 9), however, at the alveolus wall.

TABLE 2.

Percent frequency of female and male gonadal stages and number of hermaphroditic and undeterminable specimens in the period September-December 1982.

Stages	1982 Sampling Dates			
	9 Sept.	8 Oct.	19 Nov.	15 Dec.
♀♀	%	%	%	%
Fd_1	-	5.00	3.60	-
$Fd_1 + Fd_2$	-	-	32.10	31.25
$\dashrightarrow Fd_2 + Fd_3$	-	-	64.30	68.75
Fd_5/Fr_1	-	20.00	-	-
$Fr_1 + Fd_1 + Fd_2$	8.70	35.00	-	-
Fr_2*	34.80	5.00	-	-
$Fr_2 + Fr_3$*	8.70	5.00	-	-
Fr_3*	17.40	15.00	-	-
$Fr_3 + Fd_1$	30.40	15.00	-	-
♂♂	%	%	%	%
$Md_1 + Md_2$	-	-	22.70	30.00
$\dashrightarrow Md_2 + Md_3$	-	-	72.70	65.00
$\dashrightarrow Md_3 + Md_4$	-	-	-	5.00
$Md_4 + Md_5$	-	3.30	-	-
Md_5/Mr_1	9.52	10.00	-	-
$Mr_2 + Md_1 + Md_2$	-	13.30	-	-
Mr_2*	23.80	13.40	-	-
$Mr_2 + Mr_3$*	9.52	36.80	-	-
Mr_3*	19.05	10.00	-	-
$Mr_3 + Md_1$	38.11	13.30	4.60	-
♀♀	2 of 50	-	-	-
undetermined	4 of 50	-	-	-

*undetermined (undifferentiated)

Male Gonadal Growth

Male gonadal stages may be grouped in three principal phases (Table 2, Fig. 2), as in females. The onset of gametogenesis clearly occurs in many specimens from October to December. The stage of initial spermatogenesis is Md_1, in which alveoli are provided with protogonia, spermatogonia, and rare spermatocytes. Each alveolus is filled by vacuolated tissue similar to that found in females. Precocious germinal elements are mainly present at the periphery of the alveolus but also among the vacuolated cells which have penetrated the alveolus. Some of spermatogonia, indeed, migrate to occupy interstitial areas and evolve into spermatocytes entering the meiotic prophase.

In all of the September specimens and in some of the October specimens at the Md_1 stage, associated with Mr_3 ($Mr_3 + Md_1$), the vacuolated tissue is very thin, not compact. Particularly in September, at the Md_1 stage, the germinal cells are not numerous and little stained. From October, on the contrary, they are often clearly basophile and more numerous (Fig. 11). From November to December the Md_1 stage is always associated with other, more mature alveoli: $Md_1 + Md_2$ (Fig. 12), $\dashrightarrow Md_2 + Md_3$, $\dashrightarrow Md_3 + Md_4$ (Fig. 13). Sperm occupies the lumen of the alveolus in which the vacuolated cells have regressed. All of the active stages show many spermatogonia.

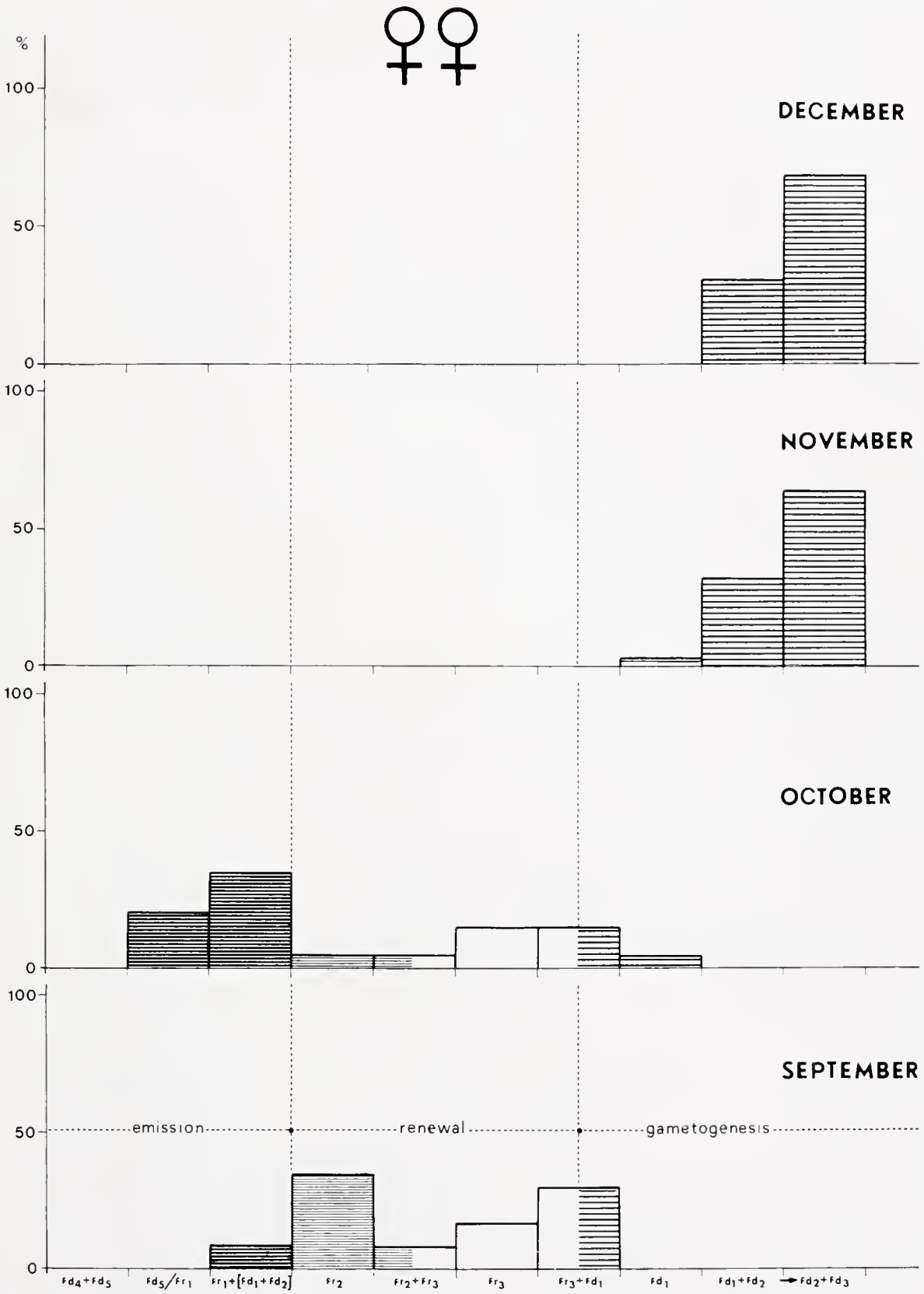


Fig. 1. Gonadal growth stages of females of *Chamelea gallina* (in %) in the period September-December 1982 (Cesenatico, Adriatic Sea).

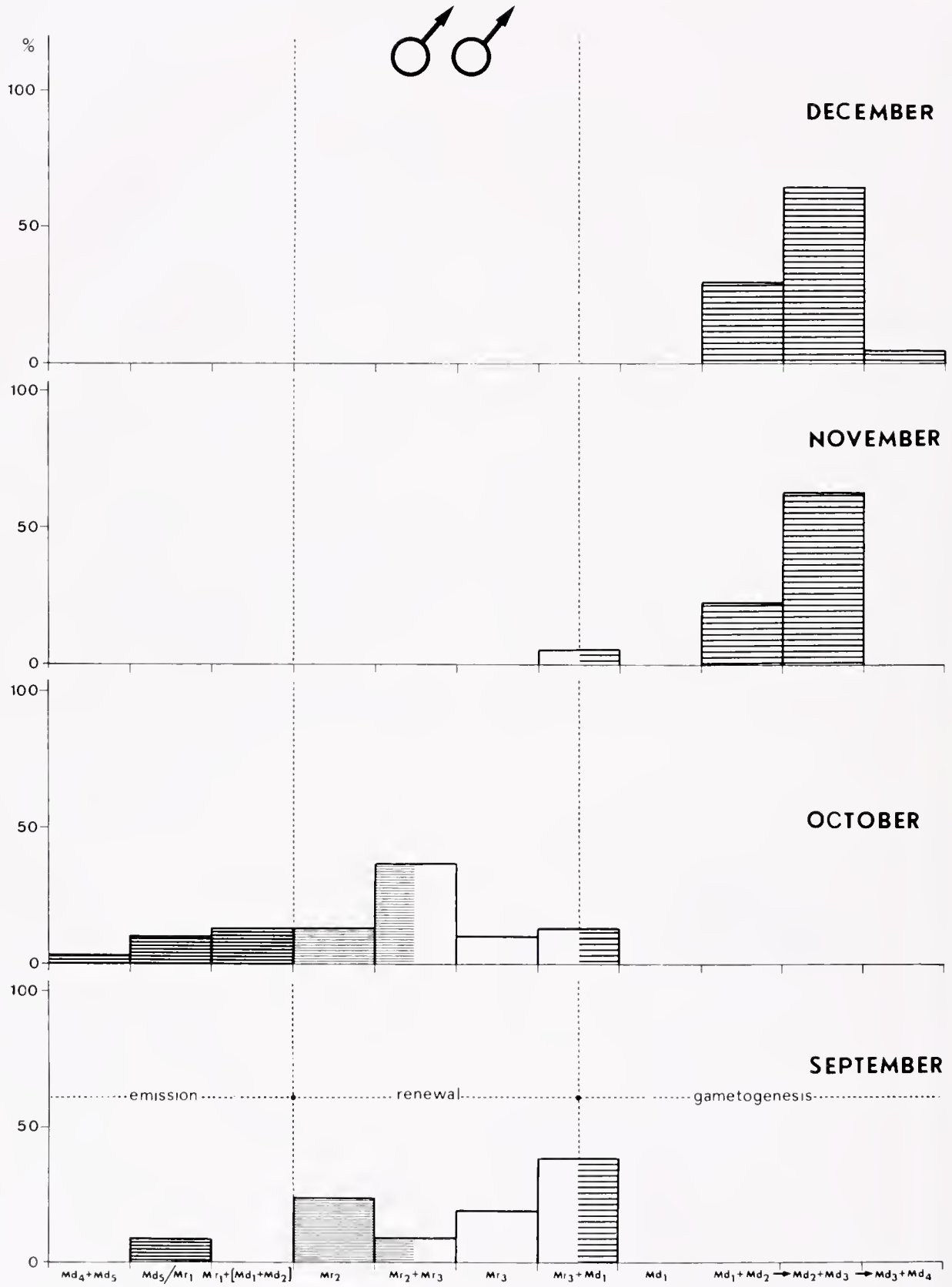


Fig. 2. Gonadal growth stages of males of *Chamelea gallina* (in %) in the period September-December 1982 (Cesenatico, Adriatic Sea).

Gamete emission occurs in September-October. Some specimens (Md_4 + Md_5) have a full gonad with almost ripe (Md_4) and ripe alveoli that are partially emitting sperm (Md_5); the gonadal wall shows few spermatogonia. Others (Md_5/Mr_1) show many ripe alveoli (Md_5) in which sperm emission (Mr_1) clearly occurs; it may be partial or almost total (Figs. 14, 15). A fraction of the specimens are at Mr_2 + Md_1 + Md_2, the gonads of which have both released sperm (Mr_2) and many alveoli that are still active (Md_1 + Md_2).

Acinus renewal occurs from September to November. This phase is characterized by stages Mr_2, Mr_2 + Mr_3, Mr_3, and Mr_3 + Md_1. Phagocytosis often occurs at stage Mr_2. The Mr_3 stage is characterized by vacuolated tissue that penetrates the thin and not compact gonad in the September specimens; the gonad gradually becomes more compact from October to November. It can be associated with the Md_1 stage, prior to gametogenesis (Fig. 16).

Hermaphroditic Specimens

Two hermaphroditic specimens were found in the September samples. One of these (SL = 26.0 mm) was classified as a functional male at the Md_5/Mr_1 stage, but small oocytes of about 20 to 30 μm in diameter were also present in some partially empty alveoli (Fig. 17). The other hermaphroditic specimen (SL = 20.0 mm) had a really mixed gonad. It was at an alveolus restoration stage and showed numerous male alveoli that were characterized by the presence of rare residual sperm; the female alveoli contained residual oocytes of 30 to 35 μm in size that occupied a well-delimited zone in a single side of the gonad. All female and male alveoli contained vacuolated tissues that were formed by large cells among which were rare degenerating oocytes and residual sperm. Many gonia were observed at the periphery. The latter specimen was classified as a Fr_3 + Mr_3 stage (Fig. 18 a, b).

In summary, the histomorphological modification of the gonad of both sexes of *Chamelea gallina* during the September-October period involved alveolus renewal in many specimens. Early stages of female and male gametogenesis were clearly present from October; on the contrary, the Fd_1 and Md_1 germinal elements in the September specimens showed low stainability. Such stages were classified as initial phases of gametogenesis because the alveolus walls, gonia, young oocytes, and spermatocytes were, at least apparently, intact; however, that they were residual or quiescent elements could not be excluded.

Gamete emission continues, after the massive summer spawning, in a small fraction of the population up to October, as noted by Guérin (1973) and Froglia (1975). Spawning can be either partial or total; its intensity is probably controlled by higher water temperatures, as indicated by periodical histological controls that were examined during 1983-1984 autumn months. Spawning appears to be an adaptive strategy of the species which includes many differentiated growth stages during the study period.

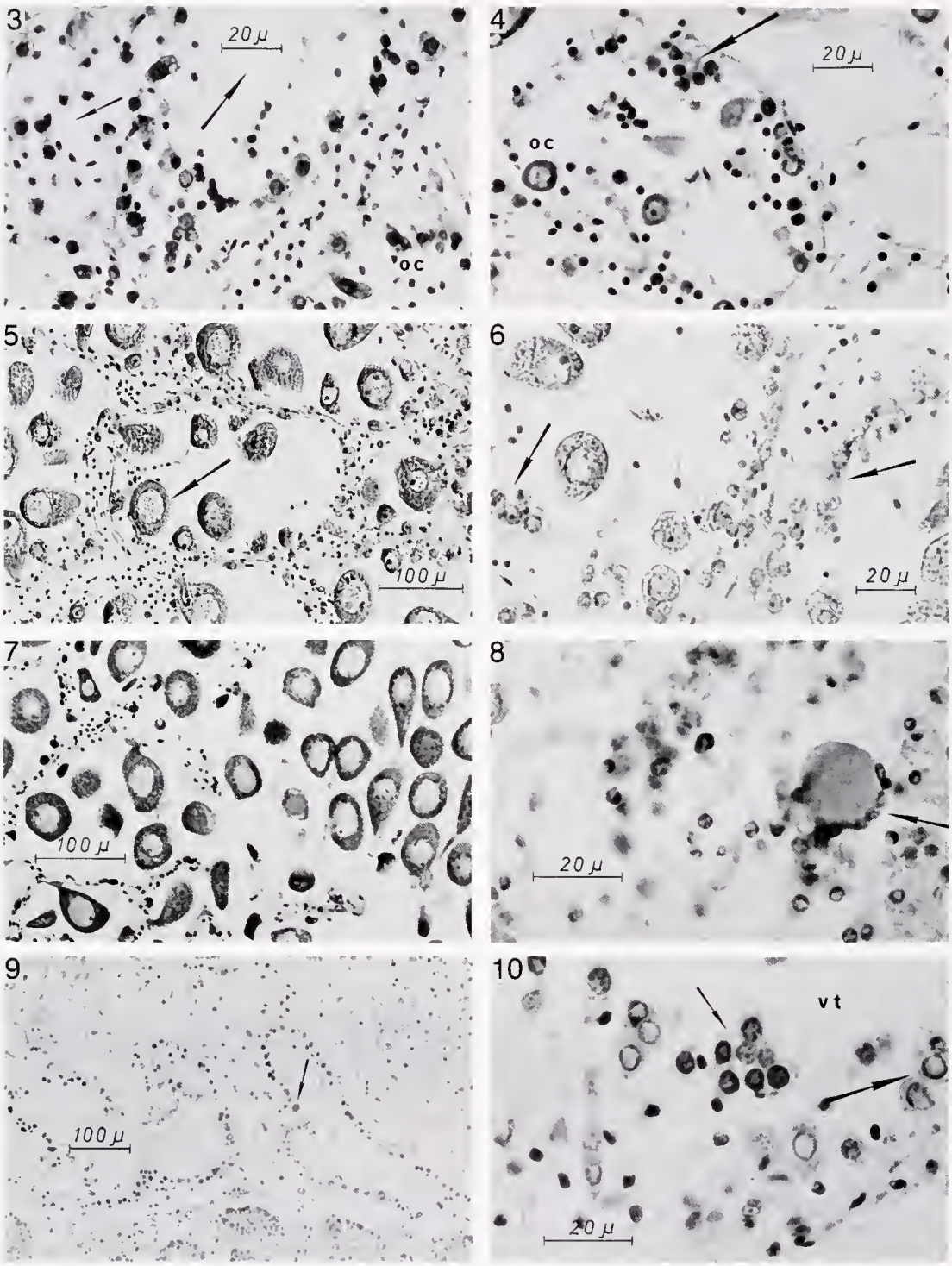
From November the gametogenetic process is active and evident in almost all of the specimens that we examined. It is differentiated among alveoli of the same gonad and also between different specimens. During November it proceeds to intermediate growth stages $\dashrightarrow Fd_3$, $\dashrightarrow Md_3$; the situation appears to be almost the same in December, but some males then show almost mature alveoli. During November a small fraction is in an alveolus restoration phase.

Active phagocytosis may occur following a total emission of gametes. The gonad of both sexes passes through an undifferentiated phase during which only somatic cells and some elements comparable to protogonia persist on the alveolus wall. The presence of such cells suggests the continuity of the germinal line during the alveolus renewal period. According to Eversole et al. (1980) undifferentiated specimens of the northern hard clam *Mercenaria mercenaria* (Linné) that were studied on a large scale, occurred more frequently among the smaller size classes (5 to 50 mm). In our samples, undifferentiated stages were particularly present in September-October specimens, without any clear clustering between age classes as defined by SL, from 20 to 30 mm.

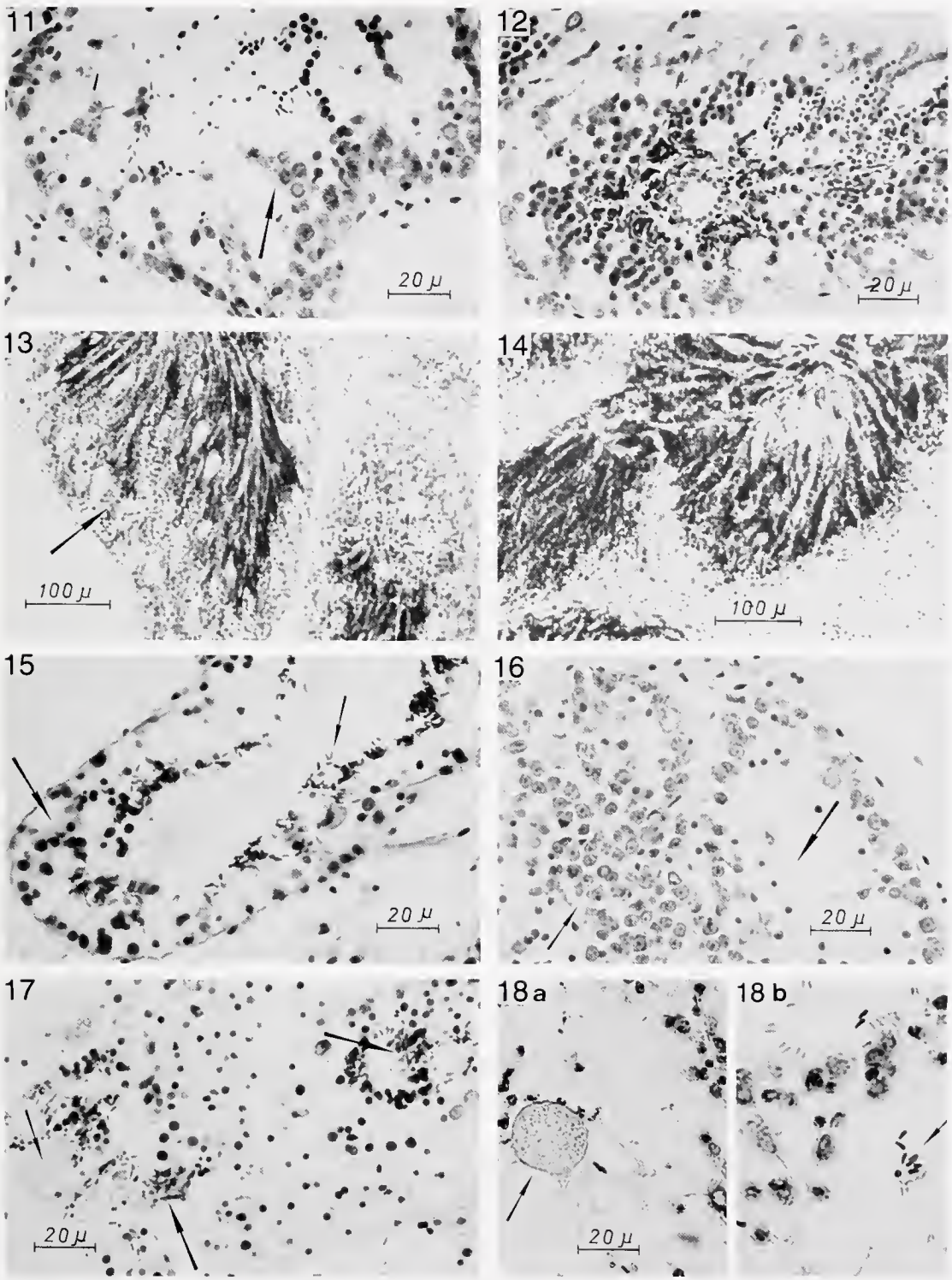
The undifferentiated phase is followed by gradual renewal of the alveolus through a tissue of vacuolated cells that penetrates the alveolus lumen from the periphery form to a sort of grating that restores the alveolus itself. That tissue is often referred to as "follicle tissue." When the alveolus is renewed gametogenesis occurs.

The first stage of female and male gametogenesis is associated with the "follicle tissue" as suggested by the contemporary presence of some composite such as Fr_3 + Fd_1 and Mr_3 + Md_1. Such a tissue precociously regresses in the ovarian alveoli and is limited to a marginal ring. In the male alveoli it stays longer and is still clearly present in the phases preceding spermiogenesis. The role of the "follicle cells" is not yet clear; some authors suggest a trophic relationship between the germinal cells and "follicle cells" (Coe and Turner 1938; Ansell 1961). According to Porter (1964) the vacuolated tissues may have a nutritive-phagocytic role. From our histological data the trophic role relative to germinal cells appears to be likely in young and in redeveloping alveoli of both sexes and particularly in specimens of the October-December samples, when the gonads are clearly active (Keck et al. 1975). A phagocytic role may also exist in phases that precede the onset of gametogenesis.

Chamelea gallina, contrary to known data, is not a rigorously gonochoric species. Two hermaphroditic specimens were noted in the 50-mm clam, September sample; one was a functional male and the other was in a restoration stage. The first may constitute a case of rudimentary hermaphroditism or a sexual inversion. The second may only suggest a case of functional hermaphroditism because the specimen was reproductively inactive. In any case, hermaphroditic specimens in this Adriatic species suggests a closer relationship with the northern hard shell clam of the Atlantic Ocean (*Mercenaria mercenaria*) from the sexuality viewpoint (Loosanoff 1937a, b). The affinity between the two species is also supported by kariological studies, since their chromosome haploid number is the same (n = 19) (Menzel and Menzel 1965, for *M. mercenaria*; Corni and Trentini 1986, for *C. gallina*).



Figs. 3-10. (3) Female: SL = 24.0 mm - Fd_1 stage - October. Cross section of two alveoli (arrows); the one on the left is penetrated by vacuolated tissue (small arrow) which, on the other hand, has regressed from the center of the other one (large arrow). Few but well stained oocytes (oc) are present. (4) Female: SL = 20.1 mm - Fd_1 stage - November. Alveolus cross section showing oogonial "nests" (arrow) and previtellogenetic oocytes (oc) ($\leq$30 μm in diameter). Note the strong basophily of the oogonia which are often engaged in mitosis. (5) Female: SL = 23.0 mm -— Fd_2 + Fd_3 stage - November. Cross section of alveoli in which oocytes at start of vitellogenesis are evident (arrow). (6) Female: SL = 23.6 mm -— Fd_2 + Fd_3 stage - December. Ovarian wall cross section: many oogonia (arrows) and oocytes at a differential growth are present. (7) Female: SL = 24.2 mm - Fd_5/Fr_1 stage - October. Residual oocytes of a spawning female (cross section). (8) Female: SL = 23.8 mm - Fr_2 stage - September. A residual oocyte (arrow) is attacked by phagocytes. (9) Undeterminable:* SL = 21.4 mm - September. On the alveolus wall there are somatic cells and cells which can be classified as protogonia (arrow). (10) Female: SL = 24.0 mm - Fr_3 + Fd_1 stage - October. Particular of an Fr_3 alveolus: vacuolated tissue (vt), protogonia (large arrow), and "nests" of oogonia are visible (small arrow).



Figs. 11-18. (11) Male: SL = 20.2 mm - Md_1 stage - October. Cross section of an alveolus penetrated by vacuolated tissue; radiating spermatogonia (arrow) are visible. (12) Male: SL: 22.0 m - Md_1 + Md_2 stage - November. (13) Male: SL = 23.6 mm -— Md_3 + Md_4 stage - December. Md_4 alveolus cross section: spermatozoa are free in the lumen; at the periphery there are very numerous radiating spermatogonia (arrow). (14) Male: SL = 22.0 mm - Md_5/Mr_1 stage - October. Partial emission of a ripe alveolus (cross section). (15) Male: SL = 21.6 mm - Md_5/Mr_1 - stage - October. Cross section of an alveolus almost empty. Residual sperm (small arrow) and a marginal ring of vacuolated cells are visible (large arrow). (16) Male: SL = 25.0 - Mr_3 + Md_1 stage - November. Cross section at the level of Mr_3 (large arrow) and Md_1 (small arrow) stages. (17) Hermaphrodite: SL = 26.0 mm - as "Md_5/Mr_1" stage - September. In some alveoli there are young oocytes (small arrow) and residual sperm (large arrow). (18) Hermaphrodite: SL = 20.0 mm - Fr_3 + Mr_3 - September. This specimen shows female alveoli with residual oocytes (arrow) (18a) and male alveoli with residual sperm (arrow) (18b).

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DENSITIES, GROWTH, AND MORTALITIES OF JUVENILES OF THE SURF CLAM (*SPISULA SOLIDISSIMA*) (DILLWYN) IN THE NEW YORK BIGHT

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ABSTRACT The objective of this study was to examine recruitment of surf clams. Bottom samples were collected with a hydraulic suction device and a Smith-McIntyre grab; and observations were made using SCUBA. Densities were nearly 2,500 clams·m^{-2} off of the northern New Jersey coast, and about 8,000 m^{-2} off of the western Long Island coast in September 1980. The clams had a mean length of 33 mm in March 1981, 6 to 8 mo after setting. By May 1981 the clams suffered 100% mortality, as a primary result of crab predation. This study and others show that surf clams set in high densities and suffer mortalities of nearly 100% every year.

KEY WORDS: Surf clam, *Spisula solidissima*, density, growth, mortality, New York Bight.

INTRODUCTION

Recent studies in nearshore waters off the coast of southern New Jersey have shown that juveniles of the surf clam *Spisula solidissima* (Dillwyn) set in dense numbers in the spring and summer, but have nearly complete mortalities by late summer and fall (Haskin et al. 1979; Garlo 1982). Nearly all the mortalities are caused by predation. It is believed that the lady crab *Ovalipes ocellatus* (Herbst), the rock crab *Cancer irroratus* Say, the common sea-star *Asterias forbesi* (Desor) (Garlo 1982) and the horseshoe crab *Limulus polyphemus* Linnaeus (Botton and Haskin 1984) are the predators. Other known predators of the surf clam are the moon snails *Lunatia heros* (Say) and *Polinices duplicatus* (Say) (Leidy 1878; Belding 1910; Franz 1977; Ropes 1980), the haddock *Melanogrammus aeglefinus* (Linnaeus) (Clapp 1912; Clarke 1954), and the Atlantic cod *Gadus morhua* Linnaeus (Bigelow and Schroeder 1953; Clarke 1954). A study in Denmark which showed high densities followed by heavy mortalities of juveniles of *Spisula subtruncata* (da Costa), with a somewhat similar pattern in 10 other juvenile bivalves (Muus 1973), suggested that temporal patterns of density and mortality might also be similar in juveniles of *S. solidissma*.

The present study examined densities, growth, and mortalities of juvenile surf clams in the New York Bight. The clams were sampled with a diver-operated suction device, and their presence was observed in grab samples. The behavior of crabs, the major predator of the clams, was examined using SCUBA gear.

MATERIALS AND METHODS

Description of Area

The study was conducted off the northern New Jersey coast and off the south coast of western Long Island (Fig. 1). Bottom sediments in the area include mud; silty-fine, fine-medium, and coarse sands; and sandy gravel.

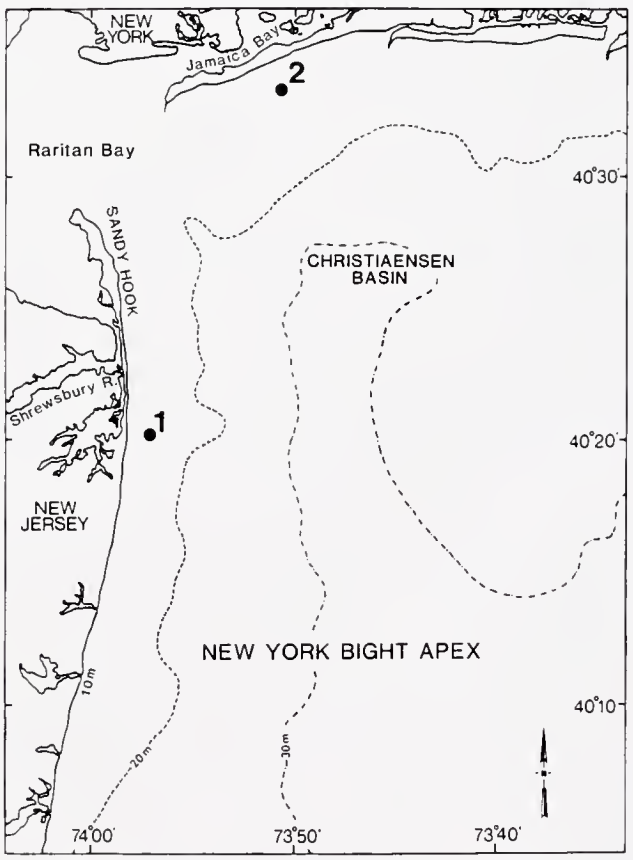

Fig. 1. Map of the New York Bight Apex showing the locations of Stations 1 and 2.

High densities of surf clams occur along the south coast of western Long Island (Meyer et al. 1981). The bottom in that area consists of fine sand and it is hard and compact. The clams

cover an area of at least 15 km^2; the mean density of clams is about 400 m^{-2}. Most clams appear to consist of a single year class, since they are uniform in size (Meyer et al. 1981). In 1982 they had a mean length of about 70 mm. A sparse population of larger clams is interspersed with the other clams.

From August 1980 to May 1981 we studied population densities and growth and mortality rates of juvenile surf clams at Station 1 (Fig. 1) by sampling at six intervals with a SCUBA-diver-operated hydraulic suction device. A ring, which encircled 0.28 m^{-2}, was tossed randomly on a sampling site, and the bottom material was sucked to a depth of about 5 cm which included all of the juvenile clams. The material was retained in a mesh bag with a mesh size of 1.5 mm, a size small enough to retain any predators and all but the recently set clams. Aboard ship, the samples were preserved in 10% buffered formalin. Four to six samples were taken on each sampling date (Fig. 2) with one exception, when adverse weather allowed only two samples to be taken. In the laboratory, the samples were transferred to isopropyl alcohol within a few days. Subsequently, the clams, clam shells, and predators were removed from the sand, counted, and measured. It was assumed that any crushed shells represented clams killed by crabs. Clam shells with a bevelled hole were assumed to have been killed by juveniles and small adults of the northern moon snail *Lunatia heros*.

We used SCUBA to make visual observations of the clams and crabs at and near Stations 1 and 2 (Fig. 1). Such observations were made at Station 1 during 1980-81 and at Station 2 during most summers from 1977-83. Each dive lasted 15 to 20 min and consisted of swimming slowly along the bottom; observations were also made while sampling with the hydraulic suction device.

From 1977-84 we sampled the invertebrates at Station 1 at intervals ranging from 4 to 12 times a year with a 0.1 m^{-2} Smith-McIntyre grab; five grabs were taken during each sampling. Any juvenile surf clams and crabs were easily seen in the grab; however, no counts were recorded.

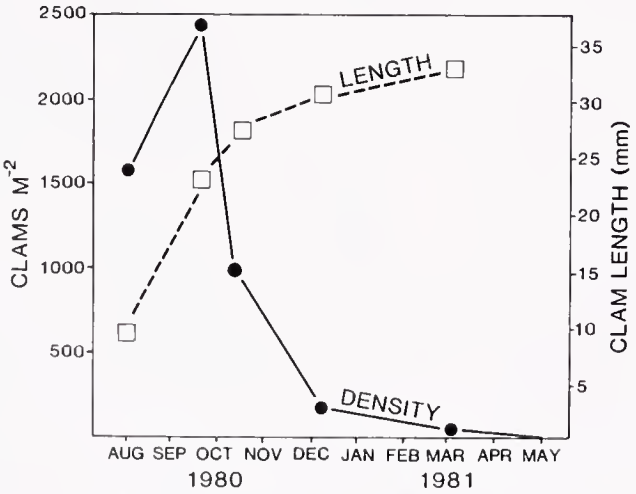

Fig. 2. The densities and mean lengths of juvenile surf clams that were collected at Station 1 during sampling dates from August 1980 to March 1981.

RESULTS

Setting Frequency and Density

From visual observations of collections made with Smith-McIntyre grabs from 1977-84, we established that juvenile surf clams settled every year at Station 1, but densities varied among years. Observations were made at Station 2 using SCUBA only, but not made every year. In whatever year they were made, however, high densities of juveniles were present.

At Station 1, the density of juvenile clams was nearly 2,500 m^{-2} in September 1980 (Fig. 2). We believe that substantial clam setting began in July, and the increase in clam density from August to September was a result of recruitment. At Station 2, we estimated from visual observations that the density of juvenile clams was about 8,000 m^{-2} in 1980.

Growth

Juvenile surf clams that were collected at Station 1 from August 1980 through March 1981 grew rapidly until early fall, and then grew slowly thereafter; they attained a mean length of 33 mm by March 1981, 6 to 8 mo after setting (Fig. 2).

Mortalities

At Station 1, juvenile surf clams suffered 100% mortality by May 1981, as shown by sampling with the hydraulic suction device (Fig. 2). Sampling with the Smith-McIntyre grab in 1980-81 and in other years showed the same mortality, but in the other years the heaviest mortalities were sometimes earlier or later than in 1980-81. Since nearly all of the dead clams had been crushed, we believe that predation by crabs was by far the most important cause of mortality; a relatively small number of clam shells had bevelled holes, an indication that the clams had been killed by the northern moon snail.

The SCUBA observations revealed that adult lady and rock crabs preyed on the juvenile surf clams. We observed that both species can crush and consume individual clams within a minute. We also collected these crabs with live and recently crushed clams in the Smith-McIntyre grab. Horseshoe crabs, which also crush clams when they feed, were rarely seen.

At Station 1, lady crabs burrowed in the coarse sand deeply enough to be covered and out of view while feeding on surf clams. We observed this while sampling clam densities with the hydraulic suction device on 3 October 1980; the crabs and clams were exposed when the sand was removed during sampling. The clam density was about 1,000 m^{-2} (Fig. 2), and it appeared that predation was heavy at the time. Each crab had the shells of perhaps 10 to 30 crushed clams near its mouth; the crabs had been reaching out and taking clams with their chelipeds without otherwise moving to any extent. Some other lady crabs were also moving quickly along the bottom, apparently looking for a place to burrow and begin feeding. We estimated that the density of lady crabs was about 3 to 4 m^{-2}. We also observed while using SCUBA that lady crabs can burrow at least 15 to 20 cm in loose, coarse sand while searching for prey. The observation was made about 2 km from Station 1, where the sand was loose enough for the diver to insert an arm vertically

into the sand up to the elbow (about 45 cm) without much effort.

We did not collect any surf clams older than juveniles at Station 1, except on one occasion when two of us found a 15 cm surf clam during a 15-min diving search.

We sampled Station 2 with the hydraulic suction device only once (18 December 1980): 48.3% of the juvenile surf clams had been killed by crabs, and 2.1% by the northern moon snail. During the years of our observations at Station 2, we observed that lady and rock crabs did not burrow into the hard bottom; they remained on the surface and excavated the juvenile clams. Moreover, the crabs did not prey on surf clams that were larger than 50 mm long at the station.

Some whole shells (paired values) of dead surf clams, 0.4 to 1 mm long, were also found in samples from Smith-McIntyre grabs in several locations in the New York Bight. Apparently, the clams died from a cause other than crab or moon snail predation.

DISCUSSION

This study and those of Haskin et al. (1979) and Garlo (1982) suggest that dense settings of juvenile surf clams occur about every year in nearshore areas along the entire coast of New Jersey and southern coast of western Long Island. Our sampling intervals were widely spaced, and thus maximum densities of juveniles were undoubtedly higher than what we recorded. The finding of high densities of juvenile surf clams was similar to that of Muus (1973) who reported densities as high as 8,500 m^{-2} of juvenile *S. subtruncata* in Denmark.

The only other reported periodic measurements of small surf clam were made in Chincoteague Inlet, Virginia (Ropes et al. 1969); the clams had a mean shell length of about 21 mm in late October and 42 mm in early July of the following year. The clams at our Station 1 had a mean length of 27 mm in late October 1980 (Fig. 2). Thus, the growth rate of small surf clams appeared to be about the same in northern New Jersey and Virginia, but we do not know whether it actually was since the times of settlement are not known. Furthermore, studies conducted in otherwise similar environments and in the same year are required to compare growth rates in different areas.

This study and those of Haskin et al. (1979) and Garlo (1982) showed as Muus (1973) had found with juvenile clams in Denmark, that mortalities of juvenile surf clams are nearly complete, at least in the studied areas. Our finding of only one large surf clam older than a juvenile at Station 1, where undoubtedly a great many had set since it was a juvenile, confirms our observations.

This study did not include observations of predation during the relatively brief period when the juveniles were less than 1.5 mm long. We do not believe that adult crabs feed on such small clams. Future investigations should examine possible predation on them. Adult lady and rock crabs and small northern moon snails were the only observed predators of 1.5 to about 33-mm juvenile clams. Observations on predation of 34 to 50-mm surf clams could not be made because they were not present. We believe that predators usually annihilate nearly all of the clams before they attain those lengths. Since 50 to 70-mm clams were not preyed upon by lady and rock crabs in the hard bottom off the southern coast of western Long Island, the clams appear to be protected from crab predation at a length of about 50 mm and perhaps somewhat smaller, at least in bottoms in which the crabs cannot easily burrow.

A large-scale predator loss appears to be the reason for an irruption in the abundance of surf clams along the southern third of the New Jersey coast. The 1976 year-class of surf clams has been relatively abundant there; based on that year-class, commerical production of surf clams has risen substantially (Murawski and Serchuk 1984). The hypoxic event that occurred off of much of the New Jersey coast in 1976 (Sindermann and Swanson 1979) killed most of the crabs and sea-stars within the affected zone (Steimle and Radosh 1979) and thus permitted clam populations to increase. Garlo (1982) found a large population of the 1976 year-class of surf clams near a coastal inlet in southern New Jersey. She also found that lady and rock crabs and sea-stars which had been abundant near the inlet in previous years had been killed or migrated from the area in 1976 as a result of the lack of oxygen. She speculated that most of the clams which set there in 1976 after the event had ended survived because the predators were scarce. Apparently, the localized phenomenon which she described occurred over the larger area.

We believe that the dense bed of surf clams off the southern coast of western Long Island formed because crabs were scarce in the area for a year or two. The clams probably set in one year in about the same density which we observed from 1979-84, but many survived instead of being annihilated by crabs as they usually are.

Crabs would have to be controlled to increase the abundance of surf clams. We can suggest a method for controlling them. Crabs can be removed from surf clam beds with modified fishing trawls or by using predator board-nets as described by MacKenzie (1979).

This study and those of Haskin et al. (1979) and Garlo (1982) considered only the juvenile surf clams which occur within about 2.5 km of shore. According to Ropes (1979), abundant surf clam populations (1 to 5 bu of clams per 4-min tow with a clam dredge having a 122-cm [48-in.] wide blade) have, at times, extended from nearshore to a distance midway across the continental shelf, or nearly 60 km from shore off the coasts of New Jersey and the Delmarva Peninsula. Future studies need to be made of densities, growth, and mortalities of juveniles in offshore areas.

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EFFECTS OF SALINITY ON SURVIVAL OF THE MSX PARASITE *HAPLOSPORIDIUM NELSONI* (HASKIN, STAUBER, AND MACKIN) IN OYSTERS

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ABSTRACT The effects of low salinity on the oyster parasite *Haplosporidium nelsoni* (MSX) were investigated by moving infected oysters from high (20-25 ‰) to 3 low salinity (5-15 ‰) locations for 4 months, then returning them to high salinity, MSX-free water. The object was to describe a time course for infection remission in low salinity, to determine whether the loss was permanent, and to observe the parasite's cytological appearance after a transfer from high to low salinity. Patent infections disappeared after 2 weeks of exposure at mean salinities of 10 ‰ or less and temperatures above 20°C. Infections did not reappear when oysters were returned to high salinity. At a mean salinity of 15 ‰ patent MSX disappeared within a month but increased again with a decrease in temperature. After 4 days of exposure to mean salinities of 10 ‰ or less, MSX plasmodia and nuclei were significantly larger than they had been at day 0, and contained very diffuse and granular cytoplasm. In contrast, the size of nuclei in cells of oyster gill epithelia remained constant in all samples taken during low-salinity exposure. An hypothesis is proposed that a physiolgical limitation may restrict the distribution of MSX in low salinity water.

KEY WORDS: *Haplosporidium nelsoni*, MSX, *Crassostrea virginica*, oyster, salinity

INTRODUCTION

Haplosporidium nelsoni (MSX) (Haskin et al. 1966; Levine et al. 1980), an acetosporan parasite of oysters, *Crassostrea virginica* (Gmelin), is prevalent in the high salinity (20-25 ‰) regions of Chesapeake and Delaware bays, but its abundance decreases regularly in an up-estuary direction (Andrews 1964, 1983; Haskin et al. 1965; Andrews and Wood 1967; Farley 1975; Haskin and Ford 1982). This pattern led to the conclusion that low salinity inhibits MSX; however, if infective stages of MSX are produced in the lower portions of each estuary, dilution of infective particles at a distance from this site would also decrease MSX prevalence in the upper estuary (Ford and Haskin 1982; Andrews 1983).

Two studies have provided experimental evidence that low salinity does, in fact, inhibit MSX. Andrews (1983) transplanted infected oysters to several locations along a salinity gradient in the James River in spring 1964 and recorded a decrease in MSX prevalence, over the following 6 to 12 weeks, at locations including and above Wreck Shoal. Salinities below 10 ‰ occurred for some period during the experiment at all locations where MSX disappeared. Sprague et al. (1969) examined salinity-MSX interactions under more controlled conditions by placing infected oysters in laboratory tanks at three salinities: 7-8, 14-16, and 19-22 ‰. After 6 months, MSX-related mortalities were 5, 26, and 88% respectively; and prevalence in survivors was 0/29, 7/14, and 1/3, respectively.

The results of both studies clearly pointed to a decline of patent MSX in oysters that were exposed to low salinity; however, because of a scarcity of histological data (Sprague et al. 1969) or of salinity data for the various stations (Andrews 1983), it was difficult to determine how quickly infections were lost at particular salinity regimes. Also, it was not clear whether the recovery was complete or whether parasite numbers were simply reduced to the point at which they were no longer detectable in the histological examination (i.e., became subpatent). In this case, they might have reappeared had the oysters been transferred to high salinity.

Reported here are the results of a field experiment in which oysters infected by MSX in lower Delaware Bay were moved to several low-salinity locations in the Maurice River (Fig. 1), left for 4 months, then returned to high salinity in an MSX-free location where chronic infections persist in imported oysters (Ford 1985). The objectives were to describe a time course for infection remission at different salinity regimes, to determine whether the disappearance was permanent, and to document changes in the cytological appearance of MSX after a change from high to low salinity.

MATERIALS AND METHODS

Oyster Movements

Oysters were transplanted from Cohansey seed bed (Fig. 1) to the planted grounds of lower Delaware Bay in May-June 1972. Infection levels and mortalities indicated that they were under very heavy disease pressure in 1972, and lighter pressure in 1973 (Ford and Haskin 1982). On 28 August 1973 survivors were suspended in trays, each containing 272 oysters, at three locations in the Maurice River (Fig. 1). The Bivalve (BIV) site is approximately 2.4 km (1.5 mi) from the river mouth. Leesburg (LEE) and Mauricetown (MAUR) are 7.2 km (4.5 mi) and 14.4 km (9 mi) upriver from Bivalve, respectively. Trays remained in the river for 4 months and were examined twice weekly for the first 2 weeks, and then at increasing inter-

vals from weekly to monthly for the remainder of the low-salinity exposure (Fig. 2). Oysters were examined histologically until no patent infections were detected and again at the end of low-salinity exposure in December 1973 (Fig. 2). On 19 December 1973 the remaining oysters (135, 117, and 124 at BIV, LEE, and MAUR, respectively) were returned to high salinity water in the following sequence: 1. overwinter storage in Cape May Harbor, where no infections occur, but where MSX persists in transplanted oysters (unpublished data, this laboratory); 2. the Cape Shore tide flats from March to May (before the summer infective period); and 3. the Shrewsbury River at Monmouth Beach, N.J., another high-salinity site where no MSX was present, but where chronic infections persist in imported oysters (Ford 1985). At Monmouth Beach, trays were sampled 5 times from June 1974 to December 1976 (Table 1).

It should be noted that native Delaware Bay oysters used in the experiment have developed a degree of resistance to MSX mortality that results in survival rates of 3 to 4 times that of imported, susceptible stocks (Haskin and Ford, 1979).

Mortality and Histological Data Collection

At each visit, dead oysters were removed from trays and those containing meat (gapers) were fixed for histology. Mortality for the interval since the previous visit was calculated as the percentage of dead oysters compared to those living at the previous sampling. Interval mortalities were accummulated to provide total mortality. Death rates of control oysters that remained on the high-salinity growing ground were estimated according to Ford and Haskin (1982).

All histological samples consisted of 20 live oysters until August 1974 after which sample size was reduced to 10 oysters. Histological processing and diagnosis were described by Ford and Haskin (1982). Prevalence of patent infections was determined for each sample and the location of parasites in each oyster was categorized as epithelial, subepithelial/local, or systemic. Infection location is a good index to intensity because parasite abundance generally increases as initial infections develop from foci in the gill epithelium into systemic infections. Also, one manifestation of resistance to MSX-mortality is an ability to prevent or delay the spread of parasites from epithelial locations (Myhre and Haskin 1970; Douglass 1977).

To quantify changes in MSX appearance after transfer to low salinity, diameters of plasmodia (the predominant stage in oysters) and nuclei were measured in oysters from each sample. In the same oysters, the nuclei of gill (plica) epithelial cells were also measured as an index to changes in host cytology.

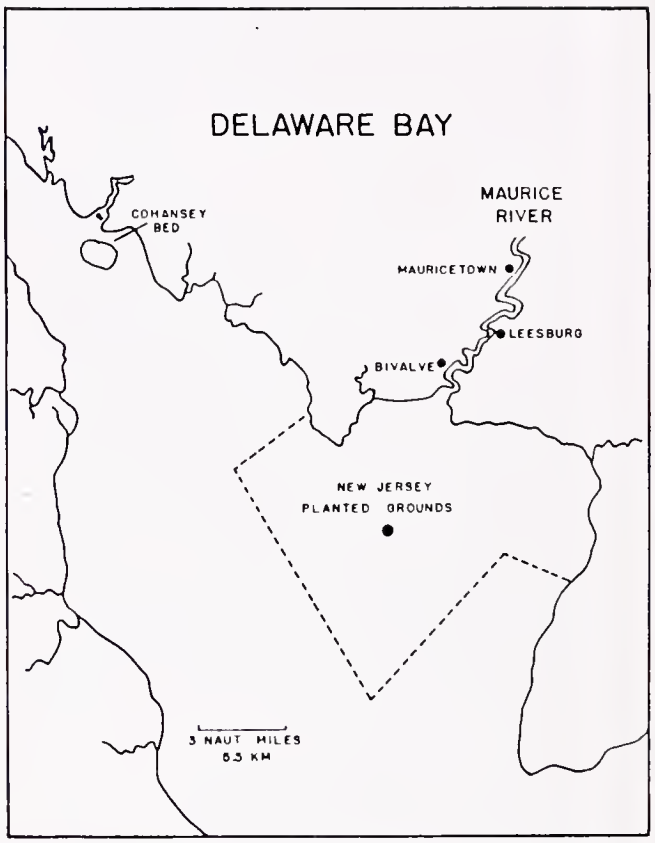

Figure 1. Delaware Bay showing low-salinity stations in the Maurice River and high-salinity control locations in the center of the planted grounds.

Salinity and Temperature

Water samples were collected several times a week at Bivalve and Mauricetown, and approximately weekly at Leesburg. Salinities were determined by silver nitrate titration, and plotted according to stage of tide. Each reading could then be standardized to an approximate midtide value using this plot. Salinities were also determined for the planted ground (high salinity control) approximately weekly as part of a continuing hydrographic program at this laboratory. Water temperatures were also taken at the Bivalve and planted ground stations at each visit. Water temperature at Bivalve was considered representative of the two upriver stations. Weekly salinity readings at Monmouth Beach between 1975 and 1977 indicated that mean salinities were generally within the range (20-25 $^{0}/_{00}$) that was favorable to MSX (Ford 1985).

TABLE 1.

Prevalences and infection intensities in low-salinity exposed oysters after they had been returned to a high-salinity, MSX-free site at Monmouth Beach.

Date	Biv	Lee	Maur
6/6/74	3/20 (M,L,R)	0/20	0/19
8/5/74	1/10 (R)	0/10	0/10
12/17/74	2/10 (H,L)	1/10 (R)	0/10
12/12/75	0/10	0/10	0/10
12/1/76	0/10	0/10	0/10

tnfection intensities are designated as H (heavy); M (moderate); L (light); and R (rare) according to Ford and Haskin (1982).

RESULTS

Salinity and Temperature

Midtide salinities at Bivalve were 13-14 $^{0}/_{00}$ during the first

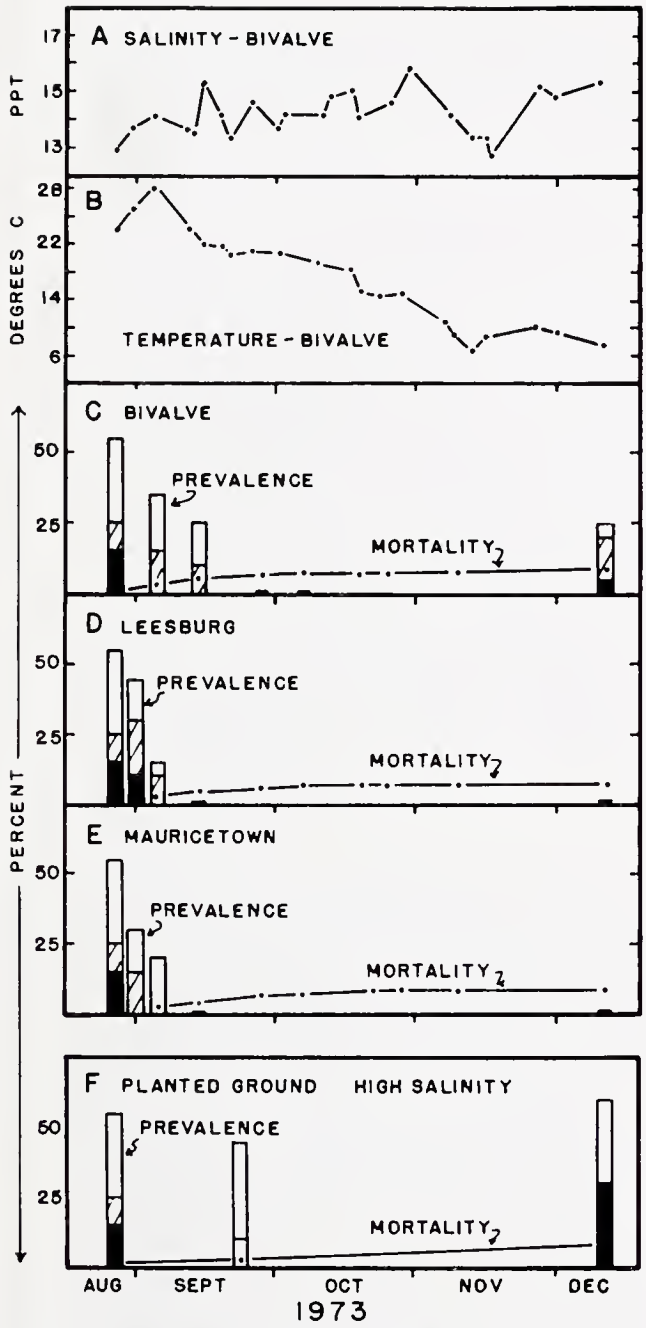

Figure 2. Midtide salinity (A) and surface temperature (B) at Bivalve. (C-D) MSX prevalence and cumulative mortality in oysters that were moved to low-salinity stations at Bivalve, Leesburg, and Mauricetown. (E) MSX prevalence and cumulative mortality in control oysters at the planted ground, high-salinity location. Prevalence bars include epithelial (open), subepithelial/local (cross-hatched), and systemic infections (solid). Histological samples were collected at days 0, 8, 16, 29, 39, and 104 at Bivalve; 0, 4, 8, 16, and 104 at Leesburg and Mauricetown; and 0, 23, and 104 on the planted ground. N = 20 for each sample.

two weeks of the experiment, then ranged between 13 and 16 $^{0}/_{00}$ until the study ended in December 1973 (Fig. 2A). At Leesburg and Mauricetown, midtide salinities were approximately 4 and 9 $^{0}/_{00}$ lower, respectively, than at Bivalve. At all stations, tidal cycle fluctuation was about 3.5 $^{0}/_{00}$ above and below the midtide value. Midtide salinities at the control location in the center of the planted grounds (Fig. 1) ranged between 21 and 23 $^{0}/_{00}$ from August to December 1973 (unpublished data, this laboratory). Surface (high water) temperatures at Bivalve were between 23° and 28°C during the first two weeks of the experiment and remained above 20°C until the end of the first week in October (Fig. 2B). Water temperatures decreased to 8-10°C during the last month of exposure.

MSX Infections and Mortalities

The MSX prevalence was 55%, although half of the infections were epithelial, at the time oysters were moved to low salinity in late August 1973 (Fig. 2C-F). In BIV oysters, prevalence decreased by half within 16 days and to zero by day 29 (Fig. 2C). Prevalence was again zero on day 39, but the final sample, taken December 10 (day 104), showed 25% of the oysters to be patently infected. The MSX disappeared more rapidly at Leesburg and Mauricetown and by day 16, patent infections were absent at both stations (Fig. 2D and E). At all locations, infection intensities decreased as prevalences declined and by day 8, no systemic infections were found (Fig. 2C-E). In samples of high-salinity control oysters, there was no significant prevalence change between August and December, although infection intensities decreased somewhat in September (Fig. 2F).

Approximately 5% of the oysters in each of the three experimental groups died during the first 2 weeks in low salinity when prevalance was decreasing. During this period, MSX was found in 2 of 4 gapers collected from each of the BIV and MAUR trays, and in 2 of 6 gapers from the LEE stock. One gaper (BIV) had a heavy infection; the rest were rare to light. There was no evidence that MSX levels declined because of deaths of infected oysters. By the end of their stay in low salinity water on 10 December 1973, total mortality was about 6% in BIV and LEE groups and 8% in MAUR. Nonpredation loss in high-salinity control oysters during the same period was 8%.

In early April 1974, after overwintering in high salinity (30-32 $^{0}/_{00}$) at Cape May Harbor, one heavily infected LEE gaper and one lightly infected MAUR gaper were found. Also, a single parasite was located in the gill epithelium of a LEE oyster in December 1974 after stocks had been at the Monmouth Beach high-salinity location for 6 months (Table 1). Other than that individual, no MSX was found in LEE and MAUR oysters during 2.5 years at Monmouth Beach. The BIV oysters continued to sustain low parasite levels at least until December 1974, but no patent infections were found after that (Table 1). At the end of the experiment in December 1976, total mortality since August 1973 was 16% in LEE and approximately 20% in BIV and MAUR groups. In contrast, control oysters under continued MSX pressure in Delaware Bay had a total mortality of 65% by May 1976.

Cytological Observations

The diameter of MSX plasmodia in MAUR and LEE samples had increased significantly ($F = 55.4$; $df = 1,53$; $p < 0.001$) from 10.8 ± 1.4 μm (mean ± 95% confidence interval) on day

0 to approximately 17.8 ± 2.1 μm on day 4 (Figs. 3A vs 3B). Parasite cytoplasm was very diffuse and granular and the nuclei also enlarged significantly ($F = 35.2$; $df = 1,202$; $p < 0.001$) from an average diameter of 3.1 ± 0.2 μm to 4.0 ± 0.2 μm (Figs. 3A vs 3B). On day 8, very few parasites were found. Of the MSX that remained, plasmodia in the LEE sample had diameters only slightly larger (12.0 ± 2.0 μm) than day 0, while MAUR plasmodia were smaller (8.8 ± 1.0 μm). The MAUR parasites were very dense, had indistinct nuclei (Fig. 3C), and resembled degenerating parasites common in late winter samples (Ford and Haskin 1982).

Cytological changes in MSX at Bivalve were much less conspicuous than at the lower salinity stations. Plasmodial size increased gradually to a mean of 13.7 ± 1.2 μm, and nuclei to 3.7 ± 0.2 μm by day 16, the last sample in which parasites were found (Fig. 3D). High-salinity oysters were sampled on day 23; plasmodia were the same diameter (10.8 ± 2.1 μm) as on day 0, but nuclei were considerably smaller (2.0 ± 0.1 μm). These parasites resembled new, rapidly proliferating plasmodia, which typically have small nuclei. In contrast to the MSX parasites, mean sizes of nuclei in gill epithelial cells ranged between 3.7 and 4.1 μm with no significant differences among samples ($F = 1.78$, $df = 7,233$; $p > 0.05$).

At Leesburg and Mauricetown, MSX nuclei were roughly spherical with inclusions or intranuclear bars (Fig. 3B); "grain-of-wheat" (Haskin et al. 1966) stages were a very small fraction of the enlarged MSX nuclei. In high-salinity control oysters, MSX nuclei were more more regular in outline, denser, and possessed peripheral endosomes (Fig. 3A & D). There was no evidence of heightened phagocytosis or other host response in any of the experimental oysters.

DISCUSSION

Results of the experiments described here demonstrated, as did those of Sprague et al. (1969) and Andrews (1983), that MSX is lost from oysters that are exposed to low salinity. The present study, however, extends the understanding of low-salinity effects on MSX by defining more precisely the timing of parasite loss at various salinity levels and by helping to suggest a mechanism for the disappearance of MSX during low salinity exposure.

Loss of parasites was progressively faster with decreasing midtide salinities from 15 to 5 ‰. At the highest salinity location, Bivalve, patent infections disappeared within a month. At both Leesburg and Mauricetown, with midtide salinities of approximately 10 and 5 ‰, respectively, the loss occurred in 2 weeks, although the intensity decrease was more rapid at Mauricetown. Andrews (1983) found that MSX prevalence in infected oysters moved to low salinity stations in the James River (including and above Wreck Shoal) in spring 1964 was reduced to zero after 6 to 12 weeks of exposure. In the same experiment, however, he found that newly patent infections from late summer 1963 exposure, which first appeared in a 5 May 1964 sample, had disappeared by 18 May, a 2-week period similar to that reported in the present experiment. Although salinities were 10 ‰ or less at some time during this period at all locations where MSX disappeared, salinity levels for individual stations were not available for correlation with infection-loss data (Andrews 1983). Thus, it is difficult to make more precise comparisons between Andrew's (1983) data and the results of the present study.

Andrews (1964, 1983) and Haskin and Ford (1982), basing their conclusions on long-term field sampling, described 10 ‰ as the lower limit for MSX survival. The rapid and virtually complete loss of MSX from live oyster samples at the Leesburg and Mauricetown stations supported this contention. Andrews (1983) concluded that low-salinity loss of MSX results from defensive activities by the oyster. This argument stemmed from his finding that the parasites disappeared at low salinity stations only when oysters resumed activity in the spring after winter dormancy. The disappearance of infections reported in the present study also occurred when oysters were active. In fact, during the period when infections were lost, temperatures were at least 20°C, a level above which defense mechanisms were thought to operate most effectively in mortality-resistant oysters (Myhre and Haskin 1970; Haskin and Douglass 1971).

While Andrews (1964, 1983) recorded low-salinity MSX disappearance in survivors of selective mortality, as did the study reported here, he also reported the same phenomenon in James River seed oysters that are very susceptible to MSX (Haskin and Ford 1979). In fact, it must be emphasized that neither the experiments reported here, nor any of the pervious studies (Andrews 1964, 1983; Sprague et al. 1969; Farley 1975; Haskin and Ford 1982), were specifically designed to differentiate between direct low-salinity kill of MSX and salinity-mediated expulsion by the host. While the latter mechanism is certainly possible, particularly in highly-resistant oysters, alternative hypotheses must also be considered.

Oysters are distributed over a wide range of salinities from about 5 to 30 ‰ (Galtsoff 1964) and are among the oligohaline osmoconformers that use amino acids to maintain a relatively constant cell volume under changing external salinities (Lynch and Wood 1966; Pierce 1971). Recent *in vitro* experiments that used trypan blue dye exclusion as a measure of cell viability have demonstrated that MSX plasmodia are considerably less tolerant of low salinity than are oyster hemocytes (Ford and Haskin [in press]). At ambient salinities of 18 to 20 ‰, about 4 to 10% of both hemocytes and MSX plasmodia absorbed dye. The proportion of stained hemocytes increased only about 2-fold when the salinity was reduced to 5-6 ‰, while the proportion of dyed MSX was approximately 80% in trials at 5 and 10 ‰. The *in vitro* results parallelled those of the present study in which enlarged MSX plasmodia and nuclei seen in histological sections of LEE and MAUR oysters contrasted with stability in the size of oyster-cell nuclei. The cytological evidence could indicate general deterioration of parasites, rather than osmotic swelling *per se*, since the size and appearance described here for parasites at low salinity are occasionally found at high-salinity stations. The *in vitro* results and the lack of any histologically detectable host response in the present ex-

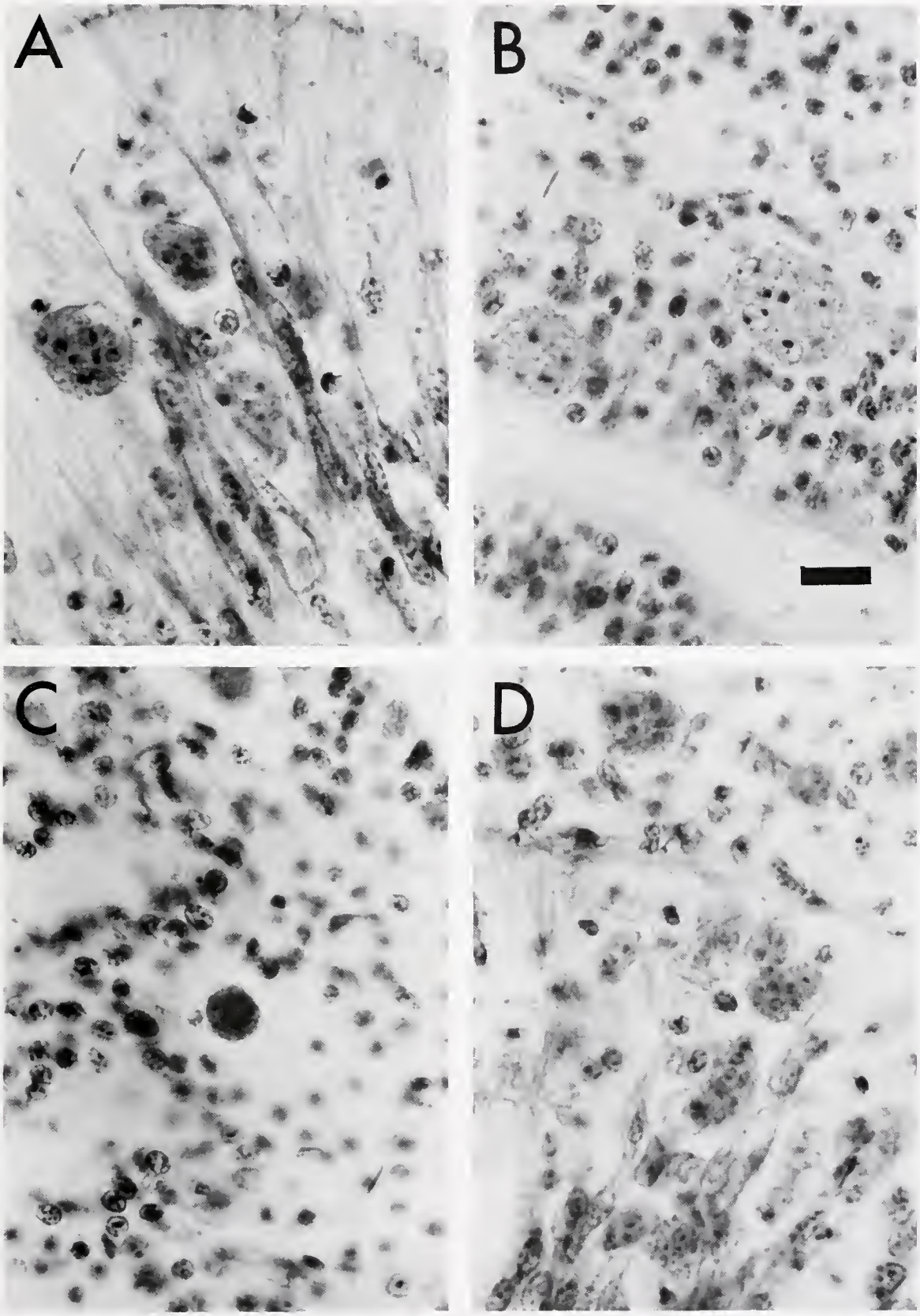

Figure 3. Cytological appearance of MSX plasmodia at high- and low-salinity locations. (A) Day 0, control (high salinity), stomach epithelium, (B) Day 4, Leesburg; gill epithelium. (C) Day 8, Mauricetown; gill epithelium. (D) Day 23, control; digestive tubule epithelium. Bar = 10 μm.

periments, however, suggest that the appearance of MSX resulted from direct salinity damage rather than from host defense activities. In fact, some physiological limitation of the parasite may be an important factor in the observed failure of MSX to become fully pathogenic at salinites between 10 and 15 $^{0}/_{00}$, as well as in its almost complete inability to tolerate salinities below 10 $^{0}/_{00}$ (Andrews 1964, 1983; Haskin and Ford 1982).

The fact that about 20% of MSX plasmodia excluded dye during acute *in vitro* exposure to low salinity (Ford and Haskin [in press]) indicates a range in the abilities of parasites to tolerate low salinity, and may explain why the MSX that survived to day 8 in LEE and MAUR oysters were not swollen, as well as why a few parasites persisted at these locations through the entire experiment.

Field studies have indicated that MSX survives, but is less pathogenic at 15 $^{0}/_{00}$ than at 20 $^{0}/_{00}$ or more (Andrews 1964; Farley 1975; Haskin and Ford 1982). The 25% prevalence at Bivalve in December, after it had dropped to zero more than 2 months earlier, suggests that new infections were acquired at this location but were retarded in development until late fall (Andrews 1964), or that there was a proliferation of MSX that had become subpatent during the first month of low salinity exposure. A resurgence of latent infections occurs in fall in high salinity ($>$ 20 $^{0}/_{00}$) areas and is believed to result from temperature-modulated host-parasite interactions that favor the parasite between 5 and 20°C (Ford and Haskin 1982; Ford 1985). The infection pattern at Bivalve may have resulted from a situation in which salinity was borderline for MSX (15 $^{0}/_{00}$) but was more damaging to the parasite at high than at low temperatures.

Metabolic changes that occur in oysters during acclimation to a large salinity change, particularly if accompanied by prolonged valve closure, might also be detrimental to the parasite. Such changes might then compound direct low-salinity damage to MSX. It can be speculated that along a gradient of progressively lower salinities, the influence of oyster activity in the loss of MSX decreases as the immediate effect of low osmotic or ionic concentration becomes an increasingly important factor in parasite destruction. Regardless of the mechanism, however, the rapid and virtually complete loss of the parasite at salinities below 10 $^{0}/_{00}$ and water temperatures above 20°C make it unlikely that MSX survives in oysters in any portion of a mid-Atlantic estuary where salinities are below 10 $^{0}/_{00}$ for 2 weeks or more during the summer. Further, it suggests a method for eliminating MSX parasites from infected oysters for experimental (Haskin and Ford 1978) or perhaps even commercial purposes by transplanting them to areas with suitably low salinity for a few weeks while oysters are metabolically active.

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PHYSIOLOGICAL EFFECTS OF THE MSX PARASITE *HAPLOSPORIDIUM NELSONI* (HASKIN, STAUBER & MACKIN) ON THE AMERICAN OYSTER *CRASSOSTREA VIRGINICA* (GMELIN).[1]

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ABSTRACT The clearance rate and condition index of *Crassostrea virginica* with systemic infections of the parasite MSX (*Haplosporidium nelsoni*) were significantly reduced compared to oysters without the parasite. No difference in the rate of oxygen consumption was found, however, between oysters with and without MSX. The depression of feeding will result in a decline in energy input which, in turn, will contribute to the significant reduction in condition index. The parasite MSX does have a measureable deleterious effect on the oyster, especially by causing a reduction in feeding rate prior to causing mortality.

KEY WORDS: Oysters, *Crassostrea virginica*, MSX, *Haplosporidium nelsoni*, clearance rate, oxygen consumption, condition index

INTRODUCTION

The oyster parasite *Haplosporidium nelsoni* (Haskin, Stauber and Mackin 1966), commonly called MSX, caused 90-95% of the oysters (*Crassostrea virginica* [Gmelin]) on planted grounds in Delaware Bay to die in the period 1957-1959 (Ford and Haskin 1982). The parasite subsequently invaded the lower portions of Chesapeake Bay in 1959 and by 1963 about 50% of the oyster beds in high salinity waters had become unproductive (Andrews 1966).

The MSX parasite appears poorly adapted to its host since infections frequently kill the oyster (Andrews 1968; Farley 1968; Ford 1985). Successful parasites will not stress their hosts fatally unless host mortality is required to release infective spores. Because the complete life cycle of the MSX parasite is presently unknown, it is possible that the oyster must die in order to release infective spores. More probably, MSX may be a very recent parasite of *C. virginica* and thus the evolution of the MSX-oyster relationship is in its early stages. The first confirmed MSX mortalities in the Delaware and Chesapeake bays occurred in 1957 (for review see Ford and Haskin 1982) and this short period of association may account for the poor degree of host-parasite adaptation (Andrews 1968). Evolutionary changes are occurring though, since native populations of oysters have started to develop some degree of tolerance to MSX in Chesapeake Bay (Andrews and Frierman 1974; Farley 1975) and Delaware Bay (Haskin and Ford 1979).

Prior investigations into the effect that MSX has on susceptible oysters prior to death, and on resistant oysters, have largely been limited to histological, biochemical, and enzymological studies of cellular disruption (Farley 1968; Feng and Canzonier 1970; Feng et al. 1970; Douglass and Haskin 1976). In order to obtain information on the physiological effects of the MSX parasite on *C. virginica*, the rates of feeding and oxygen consumption and condition indices of three separate groups of oysters were measured and each individual was then analyzed histologically for the presence of the MSX parasite.

MATERIALS AND METHODS

Experiment I

Oysters were dredged in the eastern bay region of upper Chesapeake Bay on 5 January 1983 from four localities that were thought to have a high prevalence of MSX. Ambient water temperature was 5°C and salinity was 16.5 $^{0}/_{00}$. Ten oysters from each population with shell lengths of 8-10 cm were selected, scrubbed clean, and soaked for 2 min in 0.1% solution of domestic hypochlorite (Clorox Bleach) in tap water to remove boring polychaetes (*Polydora* sp.). These oysters were maintained in a 1800-L recirculating seawater system at $16^{0}/_{00}$ and 10°C for 12 days while being continuously fed a mixture of *Isochrysis* aff *galbana* (clone T-ISO) and *Tetraselmis suecica* (Kylin) Butch to give a maintenance ration approximately equal to 3% dry tissue weight per day. The algae was added to a suspension of silt (< 32 μm) to give a 1:4 ratio by weight of organic to inorganic material which improves the oysters utilization of the algal component of the diet (see Urban and Langdon 1986).

For nine oysters from each population, the rate of oxygen consumption (ml $O_2 \cdot h^{-1}$) was measured using a radiometer oxygen electrode system (Bayne et al. 1977). The clearance rate ($L \cdot h^{-1}$) of each oyster was measured four times over a period of 8 h in a separate flow-through apparatus using a Coulter Counter to measure the particle concentration entering and leaving the experimental chamber (Bayne et al. 1977). Portions

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of the visceral mass of each oyster were prepared histologically using standard procedures (Farley 1968, 1975) to determine the degree of systemic infection by the MSX parasite. A negative diagnosis obtained by these techniques did not preclude a nonsystemic MSX infection in portions of the oyster tissue other than those sampled (Andrews 1967; Ford 1985). To account for the weight of tissue removed for histological analysis the excised pieces were wet-weighed and converted to the equivalent dry weight, using a wet weight to dry weight ratio calculated from the rest of the tissue, which was dried to constant weight at 90°C on tared pieces of aluminum foil.

Although oysters of a uniform shell length were selected, there was variation in dry tissue weight between individuals. Therefore, all values for clearance rate and oxygen consumption were corrected to a standard oyster using the allometric equation:

$$X = Y \div W^b$$

where X = weight-corrected function for a 1 g oyster,
Y = measured rate function,
W = dry tissue weight of the oyster, and
b = weight exponent of 0.65 for clearance rate and 0.7 for oxygen consumption (Newell, unpublished data).

An index of the amount of dry tissue each oyster had in relation to shell cavity size, measured by displacement, was obtained by calculating the total volume of the intact oyster (A), and the volume of the shell after the tissue had been removed (B) (Galtsoff 1964). The condition index (CI) was then expressed as:

$$CI = \frac{\text{Tissue Dry Weight}}{\text{Total Volume (A) - Shell Volume (B)}} \times 100$$

Experiment 2

Two groups of oysters were used; both were provided at the beginning of June 1983 by Jay D. Andrews, Virginia Institute of Marine Science, Gloucester Point, VA. Group 1 oysters had been collected from the low salinity Horsehead Rock oyster bar in the James River, VA, in early May, 1982 and transplanted immediately to the Gloucester Point pier, an area of high MSX disease pressure. These experimental oysters are highly susceptible to MSX (Andrews 1967; Andrews and Frierman 1974) and were expected to become infected by the parasite. Group 2 oysters came from the same Horsehead Rock populations but were collected on 5 May 1983 and held at Gloucester Point pier for approximately 26 days before being brought into the laboratory with the Group 1 oysters in June 1983. These (Group 2) oysters were the noninfected control group.

Twenty oysters with shell lengths between 8 and 10 cm from each group were cleaned as described above. They were acclimated for 17 days in the same way as described for Experiment 1 except the conditions were those ambient at Gloucester Point (salinity, 25‰; temperature, 20°C). All other experimental details and methods for measuring physiological rate functions were described for Experiment 1.

Experiment 3

A number of the Group 2 oysters were monitored by Jay D. Andrews for MSX infection using his underwater weighing technique (Andrews 1961). Andrews (1961) demonstrated that this technique can identify oysters with high levels of MSX infection which results in cessation of shell growth. Nine oysters that had ceased growing and nine that were still actively growing were collected on 8 August 1984, cleaned, and held as described for Experiment 2, except the water temperature was increased to ambient (24°C). All other experimental details and methods for measuring physiological rate functions were as described for Experiment 1.

Statistical Analysis

After the physiological data were calculated for each oyster they were combined with the results of the independent MSX analysis for that individual. This precluded bias in either the physiological or histological data analysis. Standard ANOVA techniques (SAS General Linear Models) were applied to the data from the three experiments.

RESULTS

Experiment 1

Eleven of 37 oysters from the four eastern bay populations were found to be infected with the MSX parasite. The mean ($\pm$ SE) clearance rate ($L \cdot g^{-1} \cdot h^{-1}$) of the infected group (0.30 ± 0.7) was significantly lower (Table 1; $P < 0.013$) than that of oysters without MSX (0.61 ± 0.07). Similarly, the condition index of oysters with MSX (3.79 ± 0.53) was significantly less ($P < 0.0004$) than that of the group without the parasite (6.03 ± 0.29). The rate of oxygen consumption (ml O_2 $g^{-1} \cdot h^{-1}$) was not significantly different, however, between oysters with (0.33 ± 0.29) and without (0.32 ± 0.06) the parasite.

TABLE 1.

ANOVA table for (a) clearance rate, (b) condition index, and (c) oxygen consumption data from Experiment 1.

Source	DF	SS	F	P
		(a) Clearance rate		
MSX	1	0.702	6.82	<0.013
Error	35	3.602		
		(b) Condition Index		
MSX	1	38.721	15.30	<0.0004
Error	35	88.574		
		(c) Oxygen Consumption		
MSX	1	0.0007	0.01	Not
Error	35	3.402		significant

Experiment 2

Only five of the 19 oysters from the Group 1 experimental oysters transplanted in 1982 had MSX infestations. The mean ($\pm$ SE) clearance rate ($L \cdot g^{-1} \cdot h^{-1}$) of these MSX infected oysters (1.10 ± 0.25) was significantly lower than the value of 3.03 $\pm$ 0.35 for Group 2 control oysters pooled with the noninfected

Group 1 experimental oysters (Table 2; $P < 0.043$). The condition index (4.46 ± 0.51) of the five infected oysters was also significantly below the value (5.99 ± 0.22) for the noninfected oysters ($P < 0.021$). As in Experiment 1, there was no significant difference in oxygen consumption (ml O_2 $g^{-1} \cdot h^{-1}$) between the oysters with (0.45 ± 0.23), and those without the parasite (0.56 ± 0.07).

TABLE 2.

ANOVA table for (a) clearance rate, (b) condition index, and (c) oxygen consumption data from Experiment 2.

Source	DF	SS	F	P
		(a) Clearance rate		
MSX	1	16.334	4.36	<0.043
Error	37	138.686		
		(b) Condition Index		
MSX	1	10.369	5.75	<0.021
Error	40	72.182		
		(c) Oxygen Consumption		
MSX	1	0.0496	0.25	Not significant
Error	40	8.091		

Experiment 3

Each of the two groups of nine oysters had three infected individuals, which indicated that, in this instance, the wet-weighing technique was not a particularly sensitive index of systemic MSX parasitism. A two-way ANOVA, with MSX and growth as the two independent class variables (Table 3), indicated that, in contrast to Experiments 1 and 2, the condition index of oysters with MSX (8.1 ± 1.44) was not significantly lower than that of uninfected oysters (8.38 ± 0.51). The underwater weighing technique, however, did demonstrate that the oysters that were not growing had a significantly ($P < 0.005$) lower condition index (6.84 ± 0.47) compared to oysters that were still growing (9.74 ± 0.77).

The oysters with MSX had a significantly ($P < 0.05$) depressed clearance rate ($L \cdot g^{-1} \cdot h^{-1}$; 2.26 ± 0.92) compared to the uninfected group (4.69 ± 0.64). Again, there were no significant differences in oxygen consumption (ml O_2 $g^{-1} \cdot h^{-1}$) between oysters with (1.17 ± 0.35) or without MSX (1.04 ± 0.27). There were also no differences in clearance rate or oxygen consumption between oysters that had ceased growing and those that were still actively growing.

TABLE 3.

ANOVA table for (a) clearance rate, (b) condition index, and (c) oxygen consumption data from Experiment 3.

Source	DF	SS	F	P*
		(a) Clearance rate		
MSX	1	22.88	5.03	P <0.05
Growth	1	7.89	1.74	N/S
MSX x Growth	1	3.71	0.82	N/S
Error	13	59.11		
		(b) Condition Index		
MSX	1	0.32	0.08	N/S
Growth	1	43.63	11.57	P<0.005
MSX x Growth	1	5.74	1.52	N/S
Error	14	52.79		
		(c) Oxygen Consumption		
MSX	1	0.1	0.14	N/S
Growth	1	2.04	2.77	N/S
MSX x Growth	1	0.01	0.02	N/S
Error	13	9.57		

*N/S = Not Significant

DISCUSSION

The results of three separate experiments demonstrated that the parasite *Haplosporidium nelsoni* does have a measurable, deleterious influence on the feeding rate of oysters. This is perhaps the reason why oysters with MSX have frequently been observed to have a pale digestive gland (for review see Farley 1968), which probably reflects a reduction in pigmented algal cells in the digestive system. The energy budget of MSX-infected oysters will obviously be reduced in proportion to the depressed feeding rate, as there was no compensatory reduction in metabolic rate measured as oxygen consumption. A lowered clearance rate reduces the energy available to the oyster for both germinal and somatic production and for the formation of glycogen reserves. In certain circumstances (e.g., reduced food availability, periods of high metabolic activity, etc.), these oysters will have a negative energy budget, necessitating the use of energy reserves to sustain metabolism. Both of these factors will significantly reduce the MSX-infected oysters' condition index, as was found in Experiments 1 and 2 and has been documented previously using a visual assessment of oyster condition (Farley 1968; Ford 1985). Such a cessation of growth in infected oysters is the basis of the underwater weighing technique used by Andrews (1961) to detect oysters infected with MSX. Thus, perhaps the reduced energy intake of MSX-infected oysters, combined with possible disruption of other metabolic processes associated with systemic infection, (Douglass and Haskin 1976; Farley 1968; Feng and Canzonier 1970; Feng et al. 1970; Haskin et al. 1983) may be the cause of high levels of MSX-induced mortality.

In Experiment 3 there was not a significant influence of MSX infection on condition index. In addition, the oysters determined by the underwater weighing technique to have ceased growing did not have a greater proportion of MSX than oysters determined to be actively growing. This may indicate that the MSX infections were still in the early, presystemic stages. The oysters were transplanted only 12 weeks before being measured and the parasites may not have been present for sufficient time to significantly debilitate the host. This experiment did indicate that the group of oysters which had ceased growing had a

significantly reduced condition index, which confirmed that the underwater weighing method (Andrews 1961) is sensitive to growth differences.

Bayne et al. (1978) reported that the parasite copepod *Myticola intestinalis* found in the gut of the blue mussel *Mytilus edulis* Linné also caused a significant reduction in clearance rate and this effect was somewhat proportional to the number of parasites present; however, they suggested no mechanism whereby infection by this gut parasite might reduce feeding rates. It is interesting that in neither my study nor that of Bayne et al. (1978) was there a significant effect of the parasite on the oxygen consumption of the bivalve mollusc host. The reduction in food clearance rate in these infected bivalves would not be expected to contribute appreciably to a lowering of oxygen consumption because the energetic costs of water transport are probably low in filter-feeding molluscs (Bayne and Newell 1983). *A. priori*, however, it might be expected that oysters infected with MSX would have a reduced metabolism, measured as a lowered oxygen demand, associated with the debilitating effect of the parasite. It is not known if the total metabolism of the parasites is of sufficient magnitude to compensate for any reduction in host metabolism. Further research into the influence of *H. nelsoni* on the metabolism of *C. virginica* is required to elucidate this fundamental question.

The Group 1 experimental oysters used in Experiment 2 are known to be highly susceptible to MSX (Andrews 1967; Andrews and Frierman 1974) and thus low incidence of infestation (26%) after 1 year of exposure was surprising. The histological techniques used to assess MSX infestation however will only positively identify systemic infections and may not detect localized subpatent infections (Andrews and Frierman 1974; Ford 1985). Thus, more Group 1 experimental oysters may have had MSX, perhaps localized to the gills (Andrews 1967). Therefore, the data analysis, in which Group 1 experimental oysters which were not positively identified as having MSX were combined with the Group 2 control oysters, will yield a more conservative statistical test. Any physiological differences between the MSX-infected experimental oyster and the Group 2 control oysters, collected from the same oyster bar, were minimized by the 26 days they were held together in the same field conditions followed by 17 days of acclimation to laboratory conditions.

The results from all three experiments presented here indicate that clearance rate was highly sensitive to MSX infestations. This occurred despite the large amount of variation in clearance rate as shown by the large error term in the ANOVA (Tables 1-3), which is typical for the species (Shumway 1982; Newell unpublished data). Unfortunately, because of the difficulty of obtaining sufficient oysters with different levels of MSX parasitism, I could not determine if the reduction in clearance rate was proportional to the degree of parasitism. Of the 23 oysters diagnosed to have systemic infections of MSX in these three experiments, 43.5% had levels of infection classified as *initial* (Farley 1968), 17.4% were *intermediate*, and 39.1% were *advanced* and *terminal* combined. Therefore, the deleterious effect of MSX on the clearance rate of oysters determined in this study was based on a wide range of infection intensities and not simply on the response of oysters that were terminally infected with MSX and close to death.

A number of studies have demonstrated that MSX plasmodia initially attack the oyster via the gill epithelium where they form lesions (for review see Farley 1968). From there the parasites penetrate the basement membrane, causing a sloughing of gill and palp epithelia, prior to entering the hemolymph and becoming systemic. The infection and disruption of the epithelium, and hence ciliary function of the gills, is the most likely cause for this reduction in clearance rate. The integrated functioning of the various types of cilia found on the gills of bivalve molluscs are required for the ventilatory water currents and the capture of particles (Galtsoff 1964; Jørgensen 1966). In addition, it is possible that these lesions may stimulate mucus production which will interfere with normal gill function (Jørgensen 1966). Bang and Bang (1980) demonstrated that pathogenic bacteria stimulate mucus production in the peanut worm *Sipunculus nudus* Linnaeus and physical disturbance of the gill stimulates mucus production in many bivalve molluscs (Jørgensen 1966).

Interestingly, Haskin et al. (1983) reported that oysters with systematic MSX infestations had significantly depressed protein concentrations in the hemolymph compared to lightly infected and uninfected oysters. This obviously is consistent with the results presented here because depressed levels of hemolymph proteins, as well as carbohydrates and lipids, are to be expected if the oysters' food intake is reduced; however, Haskin et al. (1983) concluded that inhibition of feeding (as indicated by digestive gland color) was not a good explanation for the low hemolymph protein.

Although the development of stocks of *C. virginica* that are resistant to MSX-induced mortality has been demonstrated (Andrews 1968; Andrews and Frierman 1974; Farley 1975; Haskin and Ford 1979), the exact mechanism conferring this resistance is not yet shown. It is clear (Farley 1975; Haskin and Ford 1979) that even so called MSX-mortality resistant oysters are still infected with the parasite, although the infection may be localized in the gills. If reduced clearance rates result from the direct effect of MSX on the gill, then even the oysters selected to be resistant to MSX mortality will have reduced clearance rates wth the consequent deleterious effects on energy intake. Therefore, future research should be designed to investigate the effect of MSX on the feeding physiology and energy balance of MSX-mortality resistant oysters.

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THE EFFECT OF VARIOUS LEVELS OF AIR-SUPERSATURATED SEAWATER ON *MERCENARIA MERCENARIA* (LINNÉ), *MULINIA LATERALIS* (SAY), AND *MYA ARENARIA* LINNÉ, WITH REFERENCE TO GAS-BUBBLE DISEASE

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ABSTRACT Supersaturated seawater was produced in a flow-through system by injecting air into a pressurized seawater line. *Mercenaria mercenaria, Mulinia lateralis*, and *Mya arenaria* were exposed to several different levels of supersaturated seawater at temperatures ranging from 5 to 17°C. Gas-bubble disease occurred at total gas saturation levels of 108% in juveniles of *M. lateralis* and 114% in juveniles of *M. arenaria*. Air blisters in the tissue, flotation, and mortality were observed at these levels. Reduced growth in juveniles of *M. mercenaria* was found at a total gas saturation level of 115%.

KEY WORDS: Gas-bubble disease, air-supersaturated seawater, *Mercenaria, Mulinia, Mya*, cultured bivalves

INTRODUCTION

Gas-bubble disease is a noninfectious disorder caused by the physical formation of gas emboli in blood and emphysema in tissues due to the uncompensated hyperbaric pressure of total dissolved gases (Bouck 1980). This disease has been described in aquactic animals by numerous authors. Weitkamp and Katz (1980) reviewed the literature on the effects of air-supersaturated water. Harvey (1975) summarized its cause and effect in fish, and Colt et al. (1984) reported gas-bubble disease in bullfrog tadpoles. This disease has also been reported in a number of invertebrates, including commercially important clams, oysters, abalone, shrimp, crabs, and lobsters, (Hughes 1968; Malouf et al. 1972; Lightner et al. 1974; Johnson 1976; Supplee and Lightner 1976; Goldberg 1978; Elston 1983).

The effect of gas bubble disease can either be acute with rapid mortality or chronic, often leading to secondary disease and gradual mortality. In bivalves it is often characterized by formation of gas blisters in soft body tissues and buoyancy of the whole animal.

Malouf et al. (1972) and Goldberg (1978) heated flowing seawater from ambient winter temperatures to about 20°C which caused supersaturation. The seawater was found harmful to the surf clam *Spisula solidissima* (Dillwyn), the bay scallop *Argopecten irradians* (Lamarck), the eastern oyster *Crassostrea virginica* (Gmelin), and the hard clam *Mercenaria mercenaria* (Linné).

Determination of dissolved gas concentrations which may affect the bivalves either acutely or chronically would be useful to the culturist, since procedures can be initiated to degas seawater to more tolerable levels. In this study the effects of gas-supersaturation on the hard clam *M. mercenaria*, the little surf clam *Mulinia lateralis* (Say), and the soft-shell clam *Mya arenaria* Linné were examined.

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MATERIALS AND METHODS

Two groups of experiments were conducted from February to March 1984 and from March to April 1985 using flowing ambient seawater pumped from Finney's Creek, Wachapreague, VA. Compressed air was introduced through a needle valve, installed on the intake (vacuum) side of the pump to supersaturate the seawater during delivery under normal pumping pressure. This supersaturated seawater was degassed in steps by cascading down a stairstep arrangement of four 19-*l* buckets with 4-cm diameter overflow pipes. Each bucket was vigorously aerated, and the overflow water fell onto a splash plate to further degas the water as it flowed into another bucket below. Each bucket had a 1.3-cm diameter drain from which seawater flowed at a rate of 4 to 10 $l \cdot min^{-1}$ into a 54-*l* polyethylene container holding the experimental animals. Water levels were held constant by a fixed standpipe. This arrangement was similar to that used by Goldberg (1978) and produced four different gas-supersaturation levels. The lowest saturation level, which was approximately the same percent saturation as the ambient water, was designated the control. Each saturation level was replicated twice.

Experimental animals were cultured at the Wachapregue laboratory and held in ambient flowing seawater prior to the experiment. One hundred *M. lateralis* and *M. arenaria* of approximately 9-mm shell height (S.H.) were held on small sieves at the bottom of each container. In a separate experiment, three groups of 100 *M. mercenaria* with mean S.H. of 5, 10, and 12 mm, and 100 *M. lateralis* of 8-mm mean S.H. were placed on sieves in test containers. In both experiments each sieve held 25 animals. Random samples of 42 *M. mercenaria* from each size class were photocopied for later measurement of initial size to the nearest 0.1 mm (Haines 1973).

Observations were made daily for 30 days and dead clams removed. Animals that floated as a result of gas bubbles in their tissues were held submerged in weighted mesh bags and checked

TABLE 1.

Gas saturation levels (mean ± standard deviation and range) used in Experiment 1, with *Mulinia lateralis* and *Mya arenaria*, and Experiment 2, with *Mercenaria mercenaria* and *M. lateralis*.

	Hyperbaric Gas Pressure mm HG	% Total Gas	% Oxygen	% Nitrogen	n
Experiment 1					
Gas Treatment					
1	149.1±30.26 (88-201)	119.6±3.97 (111.5-126.4)	116.6±6.39 (107.6-131.1)	120.6±3.87 (112.7-127.4)	13
2	103.8±18.53 (65-132)	113.6±2.42 (108.5-117.4)	111.4±4.84 (104.8-123.4)	114.4±2.38 (109.6-118.7)	13
3	58.0±11.19 (39-87)	107.6±1.48 (105.2-111.3)	105.7±3.40 (100.9-116.0)	108.1±1.58 (105.6-111.5)	13
Control	12.7± 4.36 (5-25)	101.7±0.58 (100.7-103.3)	100.1±1.24 (98.2-102.5)	102.1±0.62 (100.9-104.0)	13
Ambient	9.4±12.77 (-6.5-39.5)	101.2±1.67 (99.2-105.2)	99.1±7.32 (89.3-117.9)	101.8±1.95 (100.3-108.0)	
Experiment 2					
Gas Treatment					
1	113.3±10.39 (92.138)	114.8±1.42 (111.8-118.2)	111.0±4.00 (102.1-118.6)	116.1±1.62 (112.4-120.1)	22
2	66.5± 5.64 (57-81)	108.7±0.76 (107.3-110.6)	106.5±3.33 (101.3-114.9)	109.4±1.06 (107.1-112.6)	22
3	32.9± 4.38 (23-43)	104.3±0.58 (103.0-105.5)	102.4±1.87 (99.2-107.4)	104.9±0.63 (103.0-105.8)	22
Control	5.7± 1.40 (3-9)	100.8±0.19 (100.4-101.2)	98.4±1.55 (96.4-102.8)	101.4±0.46 (100.3-102.5)	22
Ambient	13.1± 7.05 (2-24)	101.7±0.92 (100.3-103.2)	102.6±5.56 (91.0-113.3)	101.5±0.75 (100.4-102.9)	

daily for recovery or mortality. Hard clams were held in the test containers 45 days, then transferred to flowing ambient seawater table for 5 days. Hard clams from each size class were randomly selected from the four treatments and photocopied for size determination.

Dissolved gas levels of seawater in each experimental container as well as ambient seawater were measured three or five times each week. Hyperbaric gas pressure was measured with a gasometer (Bouk 1982). Concurrent dissolved oxygen (DO) measurements were taken using the modified azide Winkler technique (APHA 1980) or a YSI (Yellow Springs Instrument Co., Yellow Springs, Ohio) Model 58 oxygen meter with a Model 5775 oxygen probe. The DO meter was air calibrated and periodic DO readings were compared to values determined from the Winkler method (Yellow Springs Instrument Co., Inc. 1982). Water temperature, salinity, and barometric pressure were measured for determination of total dissolved gas, percent oxygen saturation (% 0_2), and percent nitrogen saturation (% N_2) as described by Bouck (1982).

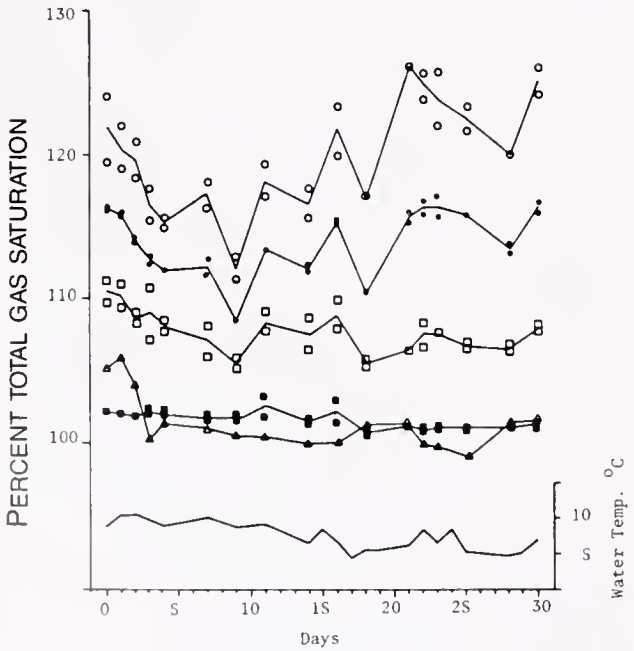

Figure 1. Percent total gas saturation recorded during Experiment 1 from each replicate for treatments 1 (O), 2 (·), 3 (□) and control (■), and for ambient seawater (Δ) with mean water temperatures.

Hyperbaric as pressure (GP) and percent total gas saturation (%TG) were compared between replicates using a t-test. Replicates were then pooled and t-tests were made to compare adjacent treatments for GP and %TG. The mean number of days of survival (Goldberg 1978) of clams in each of the replicate treatments was calculated and variances between treatment levels compared using one-way ANOVA for *M. lateralis* and *M. arenaria*. Mean number of days of survival for *M. mercenaria* was calculated in each of the eight replicate sieves and variances between gas saturation level and clam size were compared using a two-way ANOVA. Shell heights of clams were compared between replicates using a t-test. Shell height measurements of replicates were pooled for each size class and t-tests were made between treatments and between the initial measurements.

RESULTS

Means and ranges of GP, %TG, $\%0_2$, $\%N_2$, and water temperature are shown on Table 1 and Figure 1 for the first experiment using *M. arenaria* and *M. lateralis*. Fluctuations in %TG were greater at higher levels. Hyperbaric gas pressure and %TG were not significantly different between replicates; however, they were significantly different ($p < 0.001$) between saturation levels.

Means and ranges of GP, %TG, $\%0_2$, $\%N_2$, and water temperature are shown on Table 1 and Figure 2 for the second experiment using *M. mercenaria* and *M. lateralis*. Fluctuations in %TG were less intense in the second experiment than in the

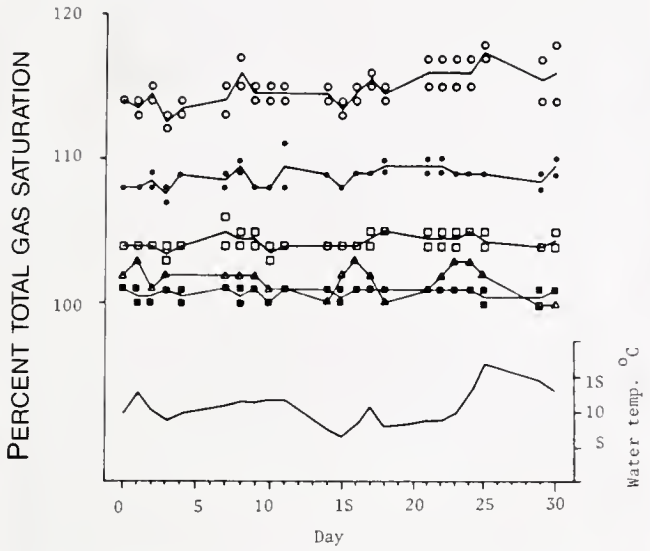

Figure 2. Percent total gas saturation recorded during Experiment 2 from each replicate for treatments 1 (O), 2 (·), 3 (□) and control (■), and for ambient seawater (Δ) with mean water temperatures.

first experiment. Significant differences ($p < 0.001$) in GP and %TG between replicates at the 112-118% and 103-106% treatment levels were indicated by t-tests. Replicates for treatment 1 had GP means and ranges of 119 mm (98-138 mm) and 108 mm (92-126 mm), respectively, and %TG means and ranges of 116% (113-118%) and 114% (112-117%). Treatment 3 replicates had GP means and ranges of 35 mm (30-42 mm) and 30 mm (23-40 mm), and %TG means and ranges of 105% (104-106%) and 104% (103-105%), respectively. Replicates within the other two treatment levels were not significantly different. The pooled replicate values between the four saturation levels for GP and %TG were significantly different ($p < 0.001$). There were no overlaps in the ranges of GP and %TG between treatment levels in the second experiment (Table 1).

Little Surf clams (*M. lateralis*) that were exposed to %TG means of 120, 114, and 108% in the first experiment were noticeably affected, with gas bubbles clearly visible in the tissues of some animals (Figure 3). Within 2 days 26% of the *M. lateralis* exposed to 120%TG floated to the surface, 5% floated at 114%TG and less than 1% floated at 108%TG (Figure 4). None of the clams in the control (102%TG) floated. Mortality of clams that were exposed to 120%TG began within 7 days with over 50% dead by day 13 (Figure 5). Survival increased with declining saturation levels. Clams that were held in low saturation (102%TG, control) showed no visible effects but those that were exposed to a %TG mean of 108% showed symptoms of gas-bubble disease. Mean number of days survival for *M. lateralis* was significantly lower ($p = 0.01$) at 120% and 114%TG (Table 2).

Little surf clams that were exposed to %TG means of 115% and 109% in the second experiment were noticeably affected, with the higher saturation level being most detrimental. Gas bubbles were also visible in the tissues of some clams. Within 3

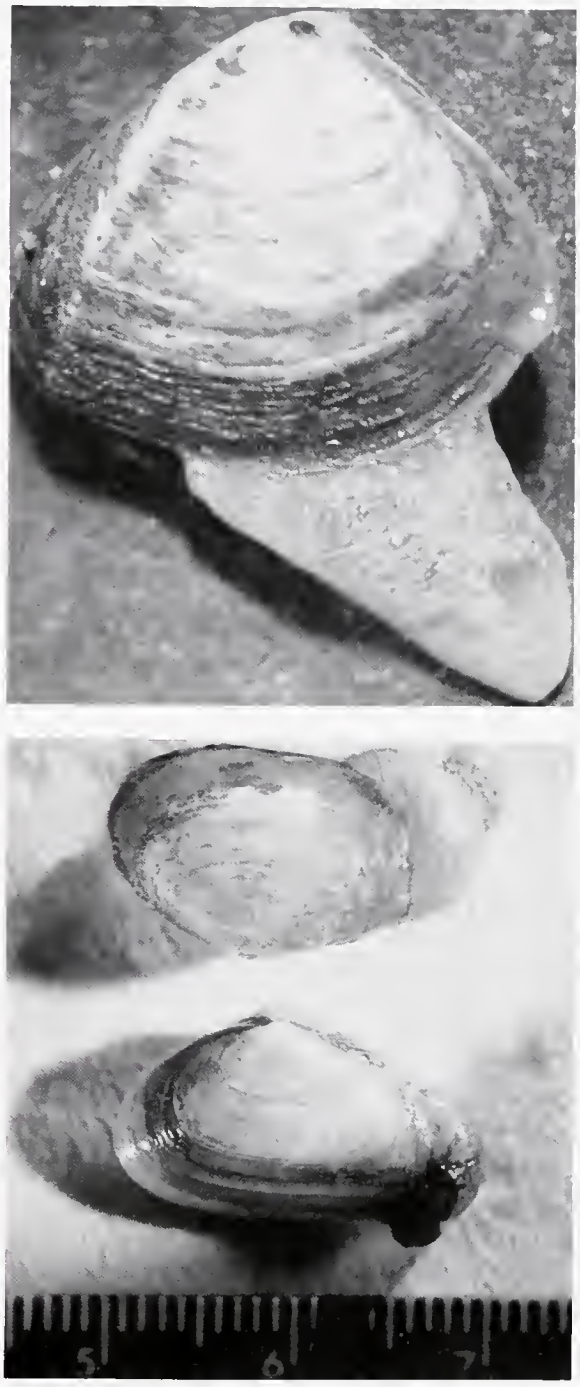

Figure 3. *Mulinia lateralis* with swollen foot caused by air bubbles in tissue (top) and *Mya arenaria* with air bubble under epidermal tissue of siphon (bottom).

days 1% of the clams that were exposed to 115%TG floated to the surface and after 10 days 96% had floated. One percent of the clams floated by day 9 in the 109%TG treatment. None of the little surf clams that were exposed to 104% or the control level (101%) were observed floating. Survival was much lower at 115%TG than at the other saturation levels (Figure 6). Exposure to 115% TG caused *M. lateralis* to begin dying within 12 days with over 50% dead by day 17. Mean number

TABLE 2.

Number days survival (mean ± standard deviation) for *Mulinia lateralis*, *Mya arenaria*, and *Mercenaria mercenaria* for each gas saturation level.

	Bivalve Species				
%TG	*M. lateralis*	*M. arenaria*	*M. mercenaria*		
120	13.0±1.70*	27.8±1.00			
114	21.6±0.13*	29.7±0.21			
108	27.8±0.40	29.7±0.39			
102 (Control)	29.5±0.31	29.7±0.24			
			5 mm	10 mm	12 mm
115	17.4±1.12*		29.4±0.48	29.5±0.44	30.0±0.08
109	29.9±0.00		30.0±0.06	29.7±0.35	29.8±0.27
104	29.7±0.16		30.0±0.00	29.7±0.75	29.9±0.31
101 (Control)	30.0±0.00		30.0±0.10	29.7±0.47	29.6±0.63

* Significantly different from control (p=0.01)

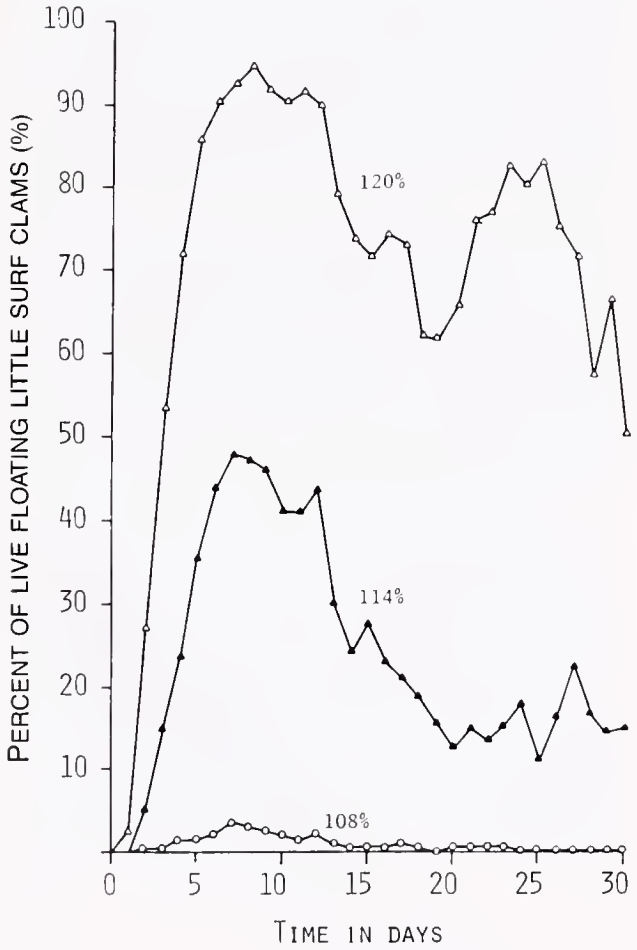

Figure 4. Percent of live little surf clams (*Mulinia lateralis*) floating for treatments having mean total gas saturation of 120%, 114%, and 108% in Experiment 1.

of days survival for *M. lateralis* was significantly lower (p=0.01) at the 115%TG (Table 2).

Mya arenaria showed a similar, though less severe, response to supersaturation. Gas bubbles developed in the body tissues

TABLE 3.

Shell heights (mean ± standard deviation) in mm of three size classes of hard clams *(Mercenaria mercenaria)* before and 50 days after exposure to gas saturation.

					n
Initial		5.4±0.42	9.7±0.65	11.5±0.76	42
After exposure					
	%TG				
	115	5.4±0.53	9.8±0.60	11.5±0.67	42
	109	6.7±0.95*	11.2±1.37*	12.8±1.20*	42
	104	6.5±0.94	10.9±1.09*	12.8±1.44*	42
	101	6.8±0.93*	10.8±1.03*	12.6±1.12*	42

*Significantly different from initial measurement (p = 0.01)

(Figure 3) and caused noticeable floatation (6%) at the 120%TG level after 4 days (Figure 7). Less than one percent of the soft shell clams floated at 108%TG and none of the control animals floated.

Some mortalities of soft-shell clams were observed after 14 days at 120%TG saturation levels (Figure 8). No significant differences in the mean number of days of survival occurred between saturation levels for *M. arenaria* (Table 2).

Mercenaria mercenaria did not appear to be adversely affected by gas supersaturation and none was observed to float. The mean number of days of survival for *M. mercenaria* was not significantly different (p=0.01) between the four saturation levels (Table 2); however, shell growth was significantly affected at 115%TG exposure for all size classes (Table 3). Shell heights of clams for replicates of the same size class were not significantly different (p=0.01). Shell heights for each size class exposed to 115% TG were not significantly different (p=0.01) from initial measurements but were significantly smaller (p<0.001) than S.H. measurements of clams from all other treatment levels. Shell heights of clams that were exposed to 109%, 104%TG, and the control (101%) were not significantly different (p=0.01) from each other for each respective size class but were significantly larger (p<0.001) than the initial measurements.

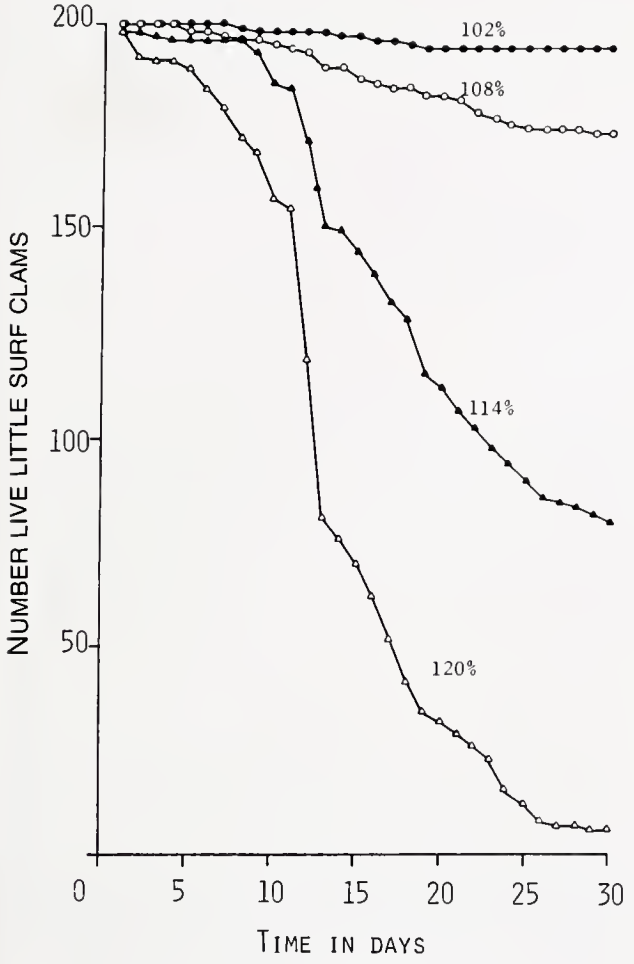

Figure 5. Survival of little surf clams (*Mulinia lateralis*) for treatments having mean total gas saturations of 120%, 114%, 108%, and 102% in Experiment 1.

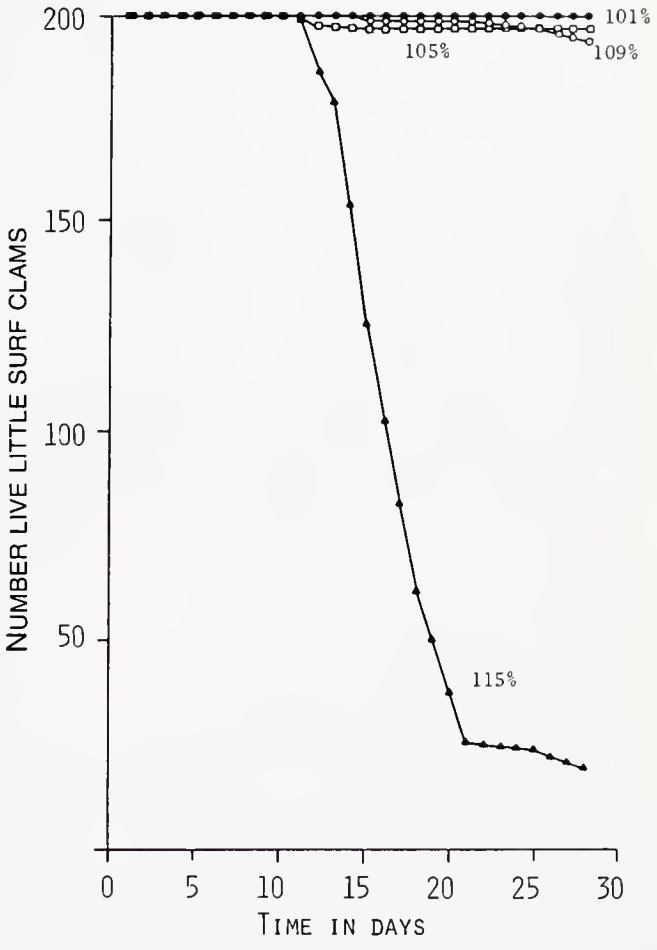

Figure 6. Survival of little surf clams (*Mulinia lateralis*) for treatments having mean total gas saturations of 115%, 109%, 105%, and 101% in Experiment 2.

DISCUSSION

Formation of air bubbles in the tissues of *M. lateralis* and *M. arenaria* as well as their observed flotation are typical symptoms of gas-bubble disease in molluscs. Increased mortality of *M. lateralis* and slower growth of *M. mercenaria* at higher saturation levels were also the effect of gas-bubble disease.

Previous studies by Goldberg (1978) indicated the detrimental effects of supersaturation at 114% O_2 and 195% N_2 in the surf clam *Spisula solidissima* and the bay scallop *Argopecten irradians* at seawater temperatures of 20°C. In this experiment gas saturation means as low as 106% oxygen and 109% nitrogen affected *M. lateralis*.

Mean hyperbaric gas pressures of 58 mm (maximum 87 mm), 104 mm (maximum 132 mm), and 113 mm (maximum 138 mm) or total gas saturation levels of 108% (maximum 111%), 114% (maximum 117%) and 115% (maximum 118%) can adversely affect small (5-12 mm S.H.) individuals of *M. lateralis*, *M. arenaria*, and *M. mercenaria*, respectively, within the temperature ranges expected during winter. An instrument such as the gasometer may be used to measure total gas pressure in culture systems for rapid and convenient monitoring of gas saturation levels.

The ability of gas-supersaturated water to cause gas-bubble disease in aquatic organisms varies between species, life-cycle stage, physiological condition, temperature, gas saturation level, and time of exposure (Goldberg 1978; Bouck 1980; Weitkemp and Katz 1980; Colt et al. 1984). The United States Environmental Protection Agency (1976) has proposed an upper water quality limit of 110% TG (GP = 76 mm). This study tends to support such criteria for juvenile molluscs.

ACKNOWLEDGMENTS

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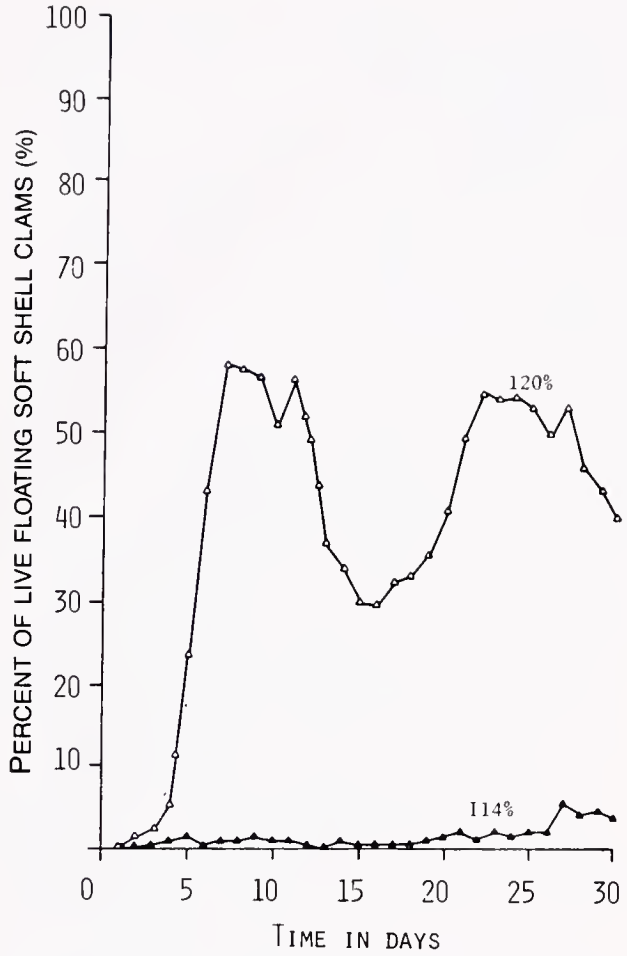

Figure 7. Percent of live soft shell clams (*Mya arenaria*) floating for treatments having mean total gas saturation of 120% and 114%.

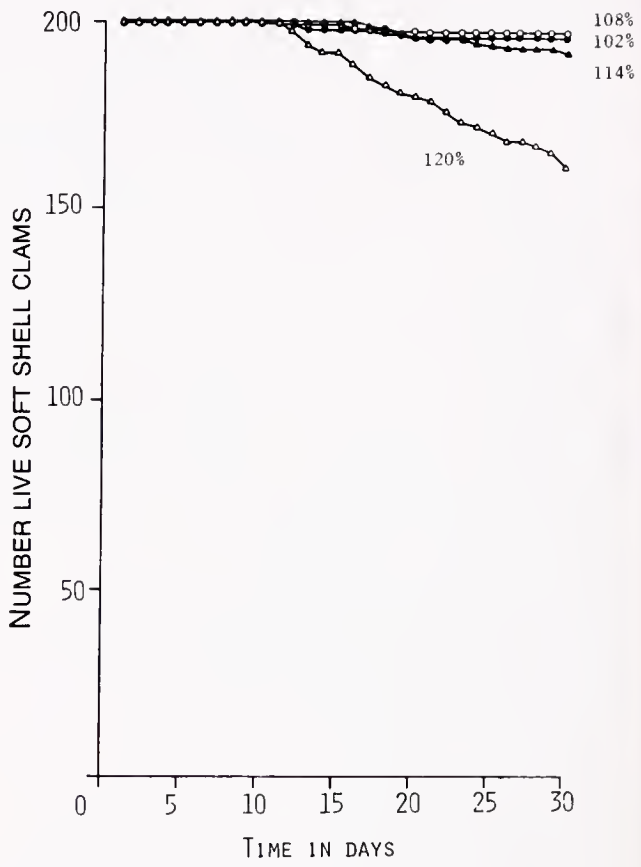

Figure 8. Survival of soft shell clams (*Mya arenaria*) for treatments having mean total aturations of 120%, 114%, 108%, and 102%.

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ESTIMATES OF NATURAL MORTALITY AND YIELD-PER-RECRUIT FOR *AMUSIUM JAPONICUM BALLOTI* BERNARDI (*PECTINIDAE*) BASED ON TAG RECOVERIES

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ABSTRACT The natural mortality coefficient, M, of the saucer scallop *Amusium japonicum balloti* in its central Queensland distribution has been estimated from the survival of tagged scallops to be approximately 0.025 week^{-1}. The analysis used to obtain this estimate of M incorporates a correction for tag shedding. The estimate of M so obtained has been incorporated in a yield-per-recruit model which allows for a seasonally varying condition factor in adductor meats. This model suggests that yield to the Queensland fishery would be maximized if age at recruitment of 28 to 36 weeks was observed. This is little different from the age at recruitment commensurate with an existing voluntary size limit of 80 to 85 mm.

KEY WORDS: Scallop, *Amusium japonicum balloti*, mortality, tagging

INTRODUCTION

The saucer scallop *Amusium japonicum balloti* Bernardi is distributed between latitudes 17°S and 27°S on the eastern Australian coast (Fig. 1), typically in water depths of 30 to 60 m. Between latitudes 21°S and 25°S the species supports a trawl fishery from which annual landings of adductor meats have averaged more than 600 tonnes in the past 5 years. Management regulations affecting the fishery are minimal and the only size limit on whole, landed scallops is a voluntary constraint on the retention of scallops with a shell height less than 80 to 85 mm by fishermen.

Information on the natural history of the saucer scallop is limited. The species is trawled from aggregations or beds which may cover areas of more than 50 km^2 (Dredge, unpub. data). These beds are separated by areas of low abundance. Gonad development commences in mid-summer, with most saucer scallops attaining sexual maturity by late autumn at the end of their first year of life (Dredge 1981). There is an inverse relationship between adductor weight and gonad weight for animals of the same shell height with adductor weight attaining a summer maximum (Williams and Dredge 1981). Spawning apparently occurs in winter and early spring, and growth of individuals is variable but rapid. Williams and Dredge (1981) have given von Bertalanffy parameter estimates for k in the range 0.051 to 0.059 week^{-1} and for L_∞ of between 102 and 109 mm shell height. From these estimates, saucer scallops with a shell height of 85 mm, which is the size at which recruitment currently occurs, are between 6 and 9 months of age. Life expectancy of saucer scallops in their Western Australian distribution is thought not to exceed 3 to 4 years (Heald and Caputi 1981).

As is the case for many species of scallop which are commercially exploited, dynamics of the stock are poorly understood. Estimates of natural and fishing mortality are not available and consequently fishing strategies can not be adequately regulated to optimize yield-per-recruit.

In this paper an estimate of natural mortality based on survival of tagged scallops together with data on growth is used to generate yield-per-recruit curves as functions of fishing mortality and age at recruitment. Estimates of optimum size at recruitment can be established from these curves and compared with the existing voluntary size limits of 80 to 85 mm (shell height).

MATERIALS AND METHODS

Field operations

To estimate the tag shedding rate for saucer scallops, multiple tagging was undertaken on two occasions. In August 1976 and October 1977 batches of 300 scallops were taken from trawl catches, triple tagged on each of the left and right valves, and released 10 to 15 km north east of Bustard Head (24°02'S, 151°46'E) in water depths of between 35 and 50 m. The tagging process, which involved glueing serially numbered Dymotape labels to scallops with an α-cyano-acrylate glue, has been described in Williams and Dredge (1981). The number and condition of remaining tags were noted for each return.

An estimate of the rate of natural mortality in the species was obtained from tagging experiments conducted between July 1977 and June 1978. Monthly releases of between 198 and 1,188 scallops were made within a 10 km radius of the original release site at 23°51'S, 151°51'E, off Bustard Head. Batches of 99 scallops, or multiples of 99, which had been tagged on both valves were released in areas where trawling was thought likely to occur in the future.

Tagged scallops were collected at approximately monthly intervals from fishing vessels and few fishermen landing scallops

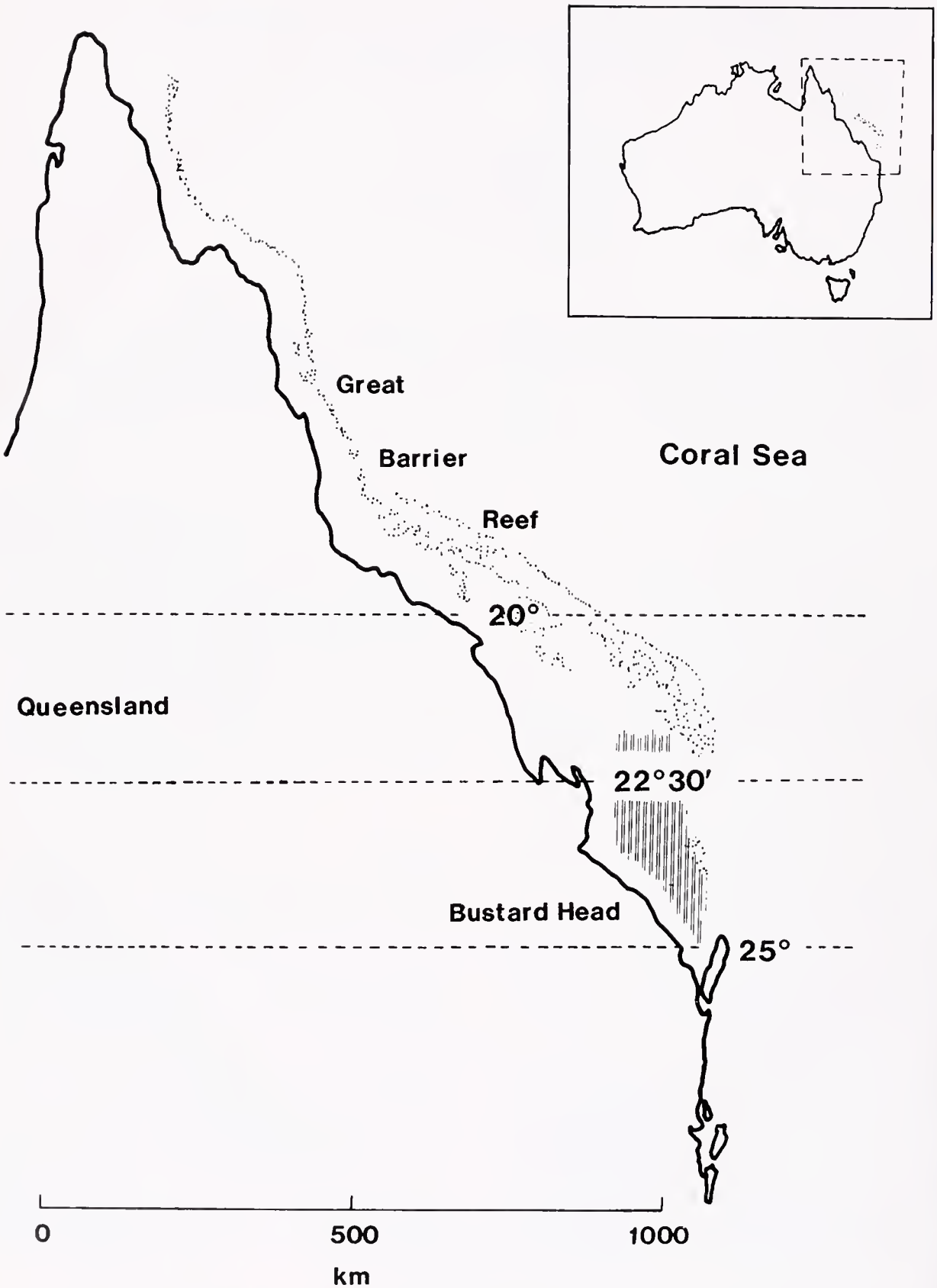


Figure 1. Northeastern Australian coastline, with fishing area for saucer scallops (hatched area).

were not contacted personally. Loss of tags through nonreporting is thought to have been negligible.

ANALYSIS

Tag shedding

As described by Davis and Reid (1982), tag shedding takes two forms: Type I shedding occurs immediately after tagging and Type II shedding is a continuous process which can be described as a rate of loss over time. Extending the analysis of Bayliff and Mobrand (1972), triple tag retention can be described in the terms

$$\ln\left(\frac{3Nxxx}{Nx + 2Nxx + 3Nxxx}\right) = \ln P - Lt_k = Y_k \quad \ldots (1)$$

where Nx, Nxx, and Nxxx are the number of scallops retaining one, two and three tags respectively on each valve in the time period centered on t_k; P is the proportion of tags retained after Type I loss; L is the instantaneous rate of tag loss; t_k the midpoint of the kth time interval; and Y is the natural log of tag retention expressed as a proportion.

Linear least-squares regression of Y_k against time, with Y_k being derived from tag returns made in a continuous series of time intervals, was used to obtain point estimates of lnP (the intercept a) and L (the regression coeffient b).

Natural mortality

Scallops are known to be sedentary after postlarval settlement (Williams and Dredge 1981) and, therefore, each released batch of tags could be treated as an isolated group whose behavior reflected that of the population as a whole. Many of the releases were fished heavily soon after release, a few were not fished at all, and in some instances, fishing took place at the point of release some time after the release took place. When fishing operations take place on a scallop bed, localized fishing mortality is heavy. Log-book data (Dredge, unpub. data) show that in some 10-min by 10-min statistical grids, catch rates have declined from 160 kg of adductor meat per boat per hour to 16 kg per boat per hour in a 14-week period, when a particularly dense bed was located and exploited by most of the fishing fleet. This implies that local instantaneous total mortality coefficient (Z) may reach a maximum of approximately 0.16 $week^{-1}$.

When a batch of tagged scallops was released but not fished for some time, the proportion of tags from the release which were returned reflected both the number which had survived, and the proportion of surviving tagged animals which had been recaptured. When fishing pressure was high, a large proportion of these surviving tagged scallops could be returned. In this case, survival in the period between release and recapture reflects that in an unfished population.

For every release of tagged scallops, an estimate of minimum survival for each week in which tag recoveries took place between the date of release and the ultimate recapture could be calculated from

$$\text{Survival (minimum)} = S_{(m)t} = \frac{N_{(\infty\text{-}t)}}{R\text{-}N_{xt}} \quad \ldots (2)$$

where $N_{(\infty\text{-}t)}$ is the number of tagged scallops returned subsequent to time t; R is number of tagged scallops released; and N_{xt} is the number of scallops lost as a consequence of tag shedding over time t. Survival over time can be converted to an instantaneous rate of survival, S, the reciprocal of which is an estimate of M, the instantaneous rate of natural mortality. Given that not all surviving tagged scallops will be caught, estimates of M that are calculated from tag returns ($\hat{M}_{max}$) will be greater than M for the species. The lowest estimates of $\hat{M}_{max}$ therefore tend toward M for the species, with error being induced by noncapture of surviving tagged scallops.

Further point estimates of $\hat{M}_{max}$ can be generated by considering losses from the original tag release due to natural mortality in the time between release and recapture. Thus

$$S(\text{min})\ (ta\text{-}tb) = \frac{N_{(\infty\text{-}tb)}}{R\text{-}N_{xta}\text{-}N_{ta}\text{-}([R\text{-}N_{xta}\text{-}N_{ta}]\cdot e^{-M(t\text{-}ta)})} \quad \ldots\ldots (3)$$

where $N_{(\infty\text{-}tb)}$ is the number of tagged scallops taken subsequent to time b; R is the number of tagged scallops released; N_{xta} is the number lost due to tag shedding up to time ta; N_{ta} is the number taken prior to time a; and $(R\text{-}N_{xta}\text{-}N_{ta})\cdot e^{-M(t\text{-}ta)}$ is the loss of tagged scallops due to natural mortality prior to time a, with $\hat{M}$ from equation (2) being substituted for M in the expression. Equation (3) should give more realistic estimates of M for the species because it reduces error by incorporating a correction for short-term natural mortality.

Yield-per-Recruit

The Beverton and Holt (1957) yield-per-recruit function is based in part upon the weight/age relationship as described by exponentiation of an integer b (the condition factor), where b usually takes the value 3. In a short lived species such as *A. japonicum balloti* which exhibits significant seasonal variation of the condition factor b (Williams and Dredge 1981), such an expression is invalid and be takes a more complex form, which may be sinusoidal (Cloern and Nichols 1978). Yield-per-recruit can be approximated using empirical estimates of weight at age in this situation. A stepwise model of this type, given by Thomson and Bell (1934) as quoted in Ricker (1976), is well suited for calculation on conventional computer spreadsheet software. In the present study weight at age was estimated from growth parameters and length/weight relationships given in Williams and Dredge (1981). For a stepwise yield-per-recruit model, yield from a given recruitment is given by

$$\text{Yield (t)} = \sum_{t_r}^{t\infty} (W_t(N_t\text{-}N_{t+1})/(F/F+M) \quad \ldots.(4)$$

where t_r is the age at which recruitment occurs; t_∞ is the age at which fishing ceases (in this model, 160 weeks); W is the average weight of adductor at time t; and N_t and N_{t+1} are the numbers remaining at times t and t+1, respectively. Growth was assumed to commence at a point interval of time (August 1st). A series of yield-per-recruit curves were generated using a point estimate for M and allowing F to vary between 0 and 0.15 $week^{-1}$ and age at first capture to vary between 24 and 52

weeks. The most rapid decline in catch rates on a given statistical block recorded in the four years between 1977 and 1980 suggested that a localized, short-term value for Z did not exceed 0.16 week^{-1}. The effect of variation in these two parameters on yield can thus be observed.

RESULTS

Tag Shedding

The numbers of tags remaining on each recaptured, triple-tagged scallop are summarized in Table 1. Mean retention of tags by the right hand valve was 2.78 tags per shell ($s = 0.49$, $n = 87$) and corresponding retention on the left hand valve was 1.83 ($s = 1.09$, $n = 87$). The difference is significant ($d = 13.19$, $p < 0.01$). Linear least-squares regressions of Y_k against time, weighted to allow for the frequency of tag returns in each time classification, were used to estimate values of lnP and L for each valve. Regression parameters are given in Table 2.

TABLE 1.

Tag retention by triple tagged scallops over time, converted to log of tag retention (Y).

Weeks of liberty	Right valve					Left valve					
	Number of tags at recapture					Number of tags at recapture					
	3	2	1	Exp	Y	3	2	1	0	Exp	Y
0 - 7.9	0			1.000	0.000	7	1		1	0.913	-0.091
8 - 15.9	3			1.000	0.000	1	2			0.429	-0.847
16 - 23.9	1	3		0.333	-1.099		1	1	2	-	-
24 - 31.9	7	3	1	0.750	-0.288	2	5	2	2	0.333	-1.099
32 - 39.9	7	2	1	0.808	-0.214	2	2	2	4	0.500	-0.693
40 - 47.9	2			1.000	0.000			1	1	-	-
48 - 55.9	37	5	1	0.910	-0.094	19	12	9	3	0.633	-0.457
56 - 63.9	5			1.000	0.000		1	3	1	-	-

Values of lnP departed significantly from zero for both regression lines ($t_{85} = 38.8$, $p < 0.001$; and $t_{74} = 6.72$, $p < 0.001$) for right and left valves, respectively, whereas values for L did not depart significantly from zero ($t_{85} = 1.70$, $p > 0.05$; $t_{74} = 0.52$, $p > 0.10$), for right and left valves, respectively. These results suggest that while Type II shedding was negligible, Type I shedding (instant loss) was significant, with 24% loss of tags on the right hand valve and 45% loss on the left hand valve. For scallops tagged with a single mark on both valves instantaneous loss of both tags thus averaged 10.8%.

TABLE 2.

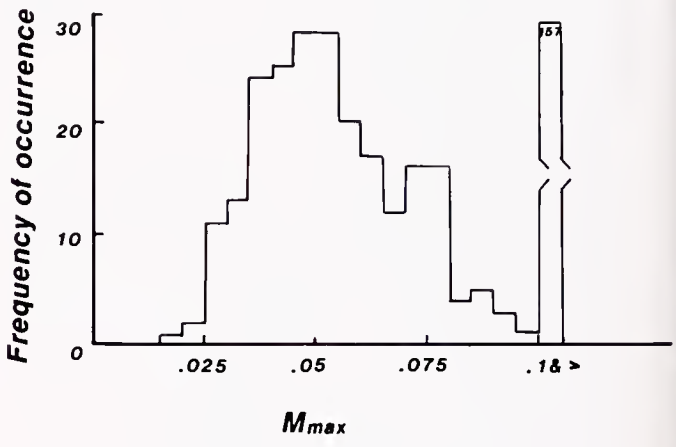
Regression parameters used to estimate tag shedding.

Value	lnp	Standard error	p	L	Standard error
Right	-0.284	0.0007	0.753	-0.0031	0.0018
Left	-0.554	0.0820	0.574	0.0001	0.0019

Estimates of Natural Mortality

In the period between July 1977 and June 1978, 56 batches of 99 tagged scallops were released off Bustard Head. Of these 1,564 (28.2%) were returned, with longest period between release and recapture being 126 weeks. Returns from individual releases ranged from 0 to 74% (Table 3). In some instances the majority of tag returns were made soon after release, but returns from other releases occurred in batches taken over short intervals of time up to two years after the date of release. Recoveries from each release were sorted into frequency of return per week of liberty, with tags being recovered in a maximum of 18 separate weeks for any one batch. Using equation (2), 393 separate estimates of $\hat{M}_{max}$ were calculated from this source and grouped (Fig. 2a). Further estimates of $\hat{M}_{max}$ calculated from equation (3) are given in Fig. 2b.

The values of M_{max} are distributed over a wide range, but for both data sets truncate sharply in the lower range of each set in the range $0.025 > \hat{M}_{max} > 0.020$ week^{-1}. For the purpose of yield-per-recruit analysis, a value of M of 0.025 week^{-1} has been adopted.



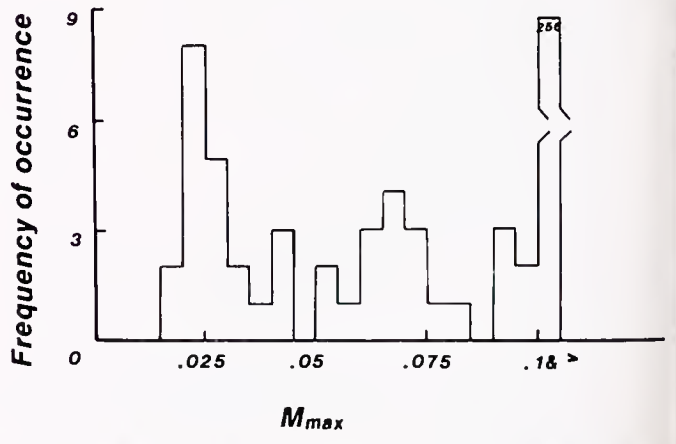


Figure 2a. Estimates of $\hat{M}_{max}$ derived from survival of tagged scallops, calculated from weekly recoveries. Figure 2b. Estimates of $\hat{M}_{max}$ derived from short-term survival data.

TABLE 3.

Number of tags returned against period at liberty.

Tag Code	Time between release and capture (weeks)																			
	0-3	4-7	8-11	12-15	16-19	20-23	24-27	28-31	32-35	36-39	40-43	44-47	48-51	52-55	56 59	60-70	71-80	81-90	91-100	101-110	>110
BEH																					
BEI																					
BEJ	25	2	29	4								2	2	3	1						
BEK	34	5	24					1			2	4		2		2					
BEL	17	1	16	1							1	8		6	1						
BRA											3			5	5				1	2	
BRB	1	3											1	2	6	1			4		
BRC		2	1	4										8					7	2	
FGA	5				1										1	6					
FGB	3		1						8	3	1	2	1								
FRA				2									30	1			5				
FRB														2	7						
FRC														1	5						
GRA	14		10								3	13	1	4	1						2
GRB	8		8								1	5	1	5	3						
GRC	3												4		1						
GRD	4	4	2										6	6	15	1					
GRE	4		2					1				1	2	4	4						
GRF												1		5							
GRG														1							
GRH	32	1																			
GRI	20	5																			
GRJ	1	35												1	3	1					
GRK	33	1									1			1	3	2					1
GRL	9			1							4	3	4			3					
GRM	1		4	1	22	4	6	9													
GRN	1			4	20	3	3	3													
GRP	30	4	2	15																	
GRQ	22	13	2	15	1																
GRR	21		22																		
GYA	13		1		1	4	1									2					
GYB	23		1		1	13	1	1								1	2				
GYC						1															
GYD		28	19	13	5									1		2					
GYE																					
GYF	5	19	13	7	4					4				1		1					
GYG		40	10	7	1			1													2
GYH	40	4	8	1					3				1	1							
ORA																					
ORE							4									2					
ORF				1												1		1			
ORG																					
ORH																					
PUA	41											2	20			1					
PUB	15	6	1							2		1	3								
PUD	28							1		5	1		6			1					
PUE	38					13				4	1	1	1								
PUF	12	1								10		1	4	7		2					
PUG												1		9	1						
PUM									3	16	2										
PUN						2	9		3	1								1			
PUO																					
REA		1													2	1	4		1		
REB	1													5	4		3	2			
REC	3			1										17		2	2				
RED	26													11	3		1		1		

Values of $\hat{M}_{max}$ that were derived from returns of triple-tagged scallops and that were calculated using values from equation (2) ranged between 0.019 and 0.957 week^{-1}. Values of $\hat{M}_{max}$ between 0.0096 and 2.27 per week were calculated from these returns using equation (3).

Yield per Recruit

Yield-per-recruit estimates from equation (4) are given in Fig. 3, with age at recruitment varying between 24 and 52 weeks (which correspond to shell heights of 75 mm and 98 mm, respectively), and varying F between 0 and 0.15 week^{-1}. These figures have been selected on the basis of their covering the full range possible in the existing fishery.

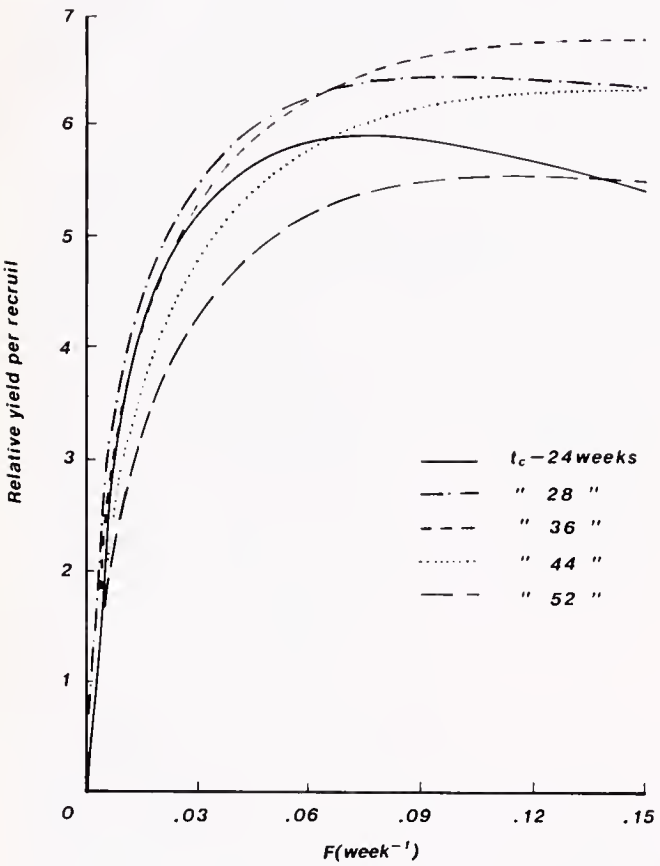


Figure 3. Yield-per-recruit.

DISCUSSION

In those fisheries for which poor statistical data bases or extreme recruitment variability in the target species preclude construction of meaningful stock-recruitment or production functions, optimization of yield-per-recruit can become a major objective in the fishery's management. This is the current status of the fishery for saucer scallops off the Queensland coast.

The purpose of this paper has been to provide an assessment of the present voluntary size limit in terms of yield-per-recruit. This assessment is dependent upon the robustness of an estimate of M for the target species. Error sources from this estimate could be expected from the noncapture of tagged scallops and from variability in tag shedding. The latter source of error only became significant when the ratio of tagged scallops to tagged scallops which had lost tags was low. This circumstance could only occur when a high proportion of tagged scallops was taken soon after release. When this occurred values of $\hat{M}_{max}$ for that release were normally in the higher range of values calculated from $\hat{M}_{max}$. Of more concern is the level of error associated with noncapture of tagged scallops.

The fishery for saucer scallops appears to exploit the resource intensively. Unpublished catch effort statistics collected in the release area during that period of the year when recruitment into the fishery was not occuring (April to November) show that between August and October 1977, catch rates declined from 46 to 27 kg·boat^{-1}·h^{-1} 1978 catch rates in the period April to November declined from 42 to 25 kg·boat^{-1}·h^{-1}. Given that some 60 trawlers, each sweeping an area of approximately 1.5 km^2·day^{-1} with an efficiency of approximately 30% (Dredge, unpub. data) worked in this area, the possibility existed for a high proportion of available tags being returned.

The validity of the value assigned to M is supported by returns of triple-tagged scallops. When values of $\hat{M}_{max}$ were calculated from this source using a tag shedding rate of 10.8 per 99 scallops released, values of $\hat{M}_{max}$ as low as .0096 were calculated. But by correcting the model to allow for the relative decrease in tag losses, estimates of $\hat{M}_{max}$ no lower than .025 were obtained.

On the basis of M being constant over the greater part of the species' life span, a value of M = 0.025 week^{-1} gives an expected survival of 2% after 3 years and 0.6% after 4 years in an unfinished population. Heald and Caputi (1981) gave an expected life span for the species of 3 to 4 years in Western Australian waters.

The yield-per-recruit curves derived from setting M at 0.025 week^{-1} show clearly the effect of increased fishing effort upon yield. For the range of t_c values examined, yield attains 90% of its maximum when F attains 0.06 week^{-1}, equivalent to an exploitation rate of 70%. As exploitation increases thereafter, yield-per-recruit either stabilizes or declines and cost-per-unit of product would certainly decline (Lucas et al. 1979).

In the lower range of fishing mortalities which have been examined, time of first capture had little effect on yield per recruit, and it is not until F values exceed 0.03 that divergent trends in yield become apparent for different values of t_c. By holding t_c to 52 weeks, yield-per-recruit appeared to attain less than 80% of the maximum attainable, whereas a t_c value of 24 weeks reduced maximum attainable yield by more than 10% when F values exceeded 0.06 week^{-1}. In the range of values for F which have been tested, yield was maintained with t_c set at 28 to 36 weeks. This is the equivalent of scallops being first fished when shell height attains 82 to 90 mm, or little different to that presently practiced in the industry.

The contrast between yield curves for *A. japonicum balloti* and the Atlantic deep-sea scallop *Placopecten magellanicus* (Gmelin) are worth noting. Yield from *P. magellanicus* is not maximized until l_c, the length at first capture, exceeds 110 mm

(Serehuk et al. 1979). This corresponds to an age of 5 to 6 years, approximately one-quarter to one-third of that attainable. Size at first capture commensurate with yield maximization for *A. japonicum balloti* is attained at a correspondingly far earlier stage in the life cycle, and, in fact, before sexual maturity is attained (Dredge 1981). While fishermen who harvest *A. japonicum balloti* have unconsciously self-regulated their behavior to maximize yield-per-recruit, it appears that the longer lead-in time for *P. magellanicus* has resulted in its being harvested at an age younger than that commensurate with an optimum l_c. Serehuk et al. (1979) suggested that l_c in 1979 was 70 to 80 mm, at which size yield was 80 to 90% of the maximum attainable over a wide range of fishing mortality rates.

While yield-per-recruit in *A. japonicum balloti* is at present being maximized, the effects of fishing the species, apparently at heavy levels, before individuals attain sexual maturity, are not fully understood. In particular, the question of stock-recruitment relations in the species needs further consideration before optimal regulation in the fishery can exist.

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